# EFFECT OF INCORPORATION OF WHEY PROTEIN PRODUCTS ON QUALITY OF BUFFALO MEAT EMULSION SAUSAGE

ABSTRACT
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# ABSTRACT

Meat and meat products are highly desired food and make important nutritional contribution to the diet of human being. Meat and its products derived from beef, lamb, pork and poultry constitute a major staple food on nutritional basis in the diet of large majority of the people in many parts of the world.

Meat and meat product are nutritionally rich, providing a wide range of nutrients, such as protein, fat, minerals and vitamins and constitute an important part of the European diet (Cosgrove *et al.,* 2005). Meat has long been considered a highly desirable and nutritious food, and has become a mass consumer product throughout the world with the highest consumption rates being recorded in industrialized Western countries. Meat is a very versatile culinary product and has become a vital element of both cuisine and culture.

Meat and meat products make an important nutritional contribution to the diet of the people. A significant percentage of the recommended dietary allowances for proteins, vitamins- B, magnesium, iron and zinc are contributed by red meat and poultry (Pearson and Brooks, 1978). Meat is also highly desired food, which fat, protein, minerals and a small proportion of carbohydrate. It is recognized as a highly nutritious food being an excellent source of high quality protein, containing a well balance of the essential amino acids and having high biological value. Meat also contributes a significant percentage of number of other minerals including sodium, potassium and magnesium.

The world buffalo population is estimated at 185.29 million head, spread in some 42 countries, of which 179.75 million (97%) are in Asia (FAO, 2008). During the last 10 years, the world buffalo population increased by approximately 1.49% annually, by 1.53% in India, 1.45% in Asia and 2.67% in the rest of the world. World livestock species related cattle and buffalo respectively 58.5 and 3.1 (FAO, 2012).

India has a major show in world buffalo population; Pakistan and China are second and third in the world of the buffalo population. India has 105.1 million and they comprise approximately 59 percent of the total world buffalo population, while Pakistan and China have respectively 29.0 million and 23.27 million (Badpa and Ahmad, 2014; Ramesh, 2013).

The meat production has increased from 764,000 tons in 1970-71 to 6.27 million tons in 2010. The compounded average growth rate (CAGR) during the last two decades works out to be 4.5%. It is noticed that about 10.6% cattle, 10.6% buffaloes, 24.1% sheep, 58.7% goats, 95.0% pigs and 190.0% chicken are slaughtered each year (Meat trade news, 2012).

The present production of meat from India is estimated at 9.29 million tons in 2011 (FAO, 2012), which is 2.21% of the world's meat production. The contribution of meat from buffalo is about 17.0%, while cattle 16.0%, sheep 3.0%, goat 6.0%, pig 6.4% and poultry 51.0%. Looking into the carcass weight per animal in the country is much below the world average in cattle and where as reaching to the world average in buffalo, sheep, and goat. About 33% of meat productions in the country are from cattle and buffalo; about 6% from goat whereas about 3% is produced from sheep (Mane, 2012).

Indian buffalo meat production is growing significantly. Calendar year (CY) 2013 Indian buffalo meat production is thus forecast to rise to a record 4.16 million tons (on a carcass weight equivalent basis), up 14 percent from CY 2012. CY 2012 buffalo meat production is estimated at 3.64 million tons (up 12% from CY 2011), and CY 2011 production has been slightly revised up to 3.24 million tons (Agricultural and Processed Food Products Export Development Authority, 2013).

Buffalo meat can be very well used for production of sausage, a ready to eat and serve product. Sausage is a food that is prepared from comminuted and seasoned meat and is usually formed into a symmetrical shape. The word sausage comes from the Middle English sausage, which Latin Salsus, which means salted or literally meat preserved by salting. In France they are sausissons and in Germany, Wurst. In practice for over a millennia sausage-making was originally a method used to preserve meats, especially lesser cuts (Marianski and Marianski, 2008).

The emulsion sausages are that have been finely comminuted to the consistency of a fine paste. Hot dog, Frankfurter, Mortadella, Bologna, liver sausage is typical examples. Emulsion sausage will be successful if the enough lean meat has been selected and enough myosin has been extracted. The lean meat is the main source of myosin. The more myosin extracted, the thicker and stronger protein coat develops around particles of fat. In cause of myosin depends on how vigorous the cutting process was and how much salt (and phosphates) was added.

Whey proteins are by-products of the chesses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Now a day, great efforts are being made to find new uses for whey proteins, e.g. production of edible film (Anker *et al.*, 1998). Whey protein improves emulsion stability, provide better color properties and result in lower chewiness and elasticity(Yetmin *et al.,* 2001). Whey protein consists of a number of individual protein components. The two most abundant proteins are β-Lac (50-55%) and α-Lac (20-25%).

Whey protein is a high quality, complete protein with all the essential amino acids. Whey protein is richest source of naturally occurring branched chain amino acids (leucine, isoleucineand valine). These are important for active individuals, exercise and professional athletes. Adding whey protein to the diet is great ways to jump start a weight loss program. Whey protein is a key ingredient in numerous weight loss and meal replacement products and whey protein isolate (with no fat or carbohydrates).

Emulsion sausage incorporated with whey protein products is one product, which can be prepared from buffalo meat using other ingredient salt, spices, condiment, whey protein (isolate, concentrate, whey powder), fat and animal fat. The product will have a pleasant taste, excellent flavour and increased juiciness. Also product contains bioactive ingredients like immuglobulins and lactoferrin, which help to support the immune system. Emulsion sausage incorporated with whey protein products will have rich abundance of branched chain amino acids and its quick absorption rate. These are important to help repair and rebuild muscles. Emulsion sausage treated with whey protein products have fresh, natural taste and do not contain isoflavones and any other component with potential hormonal effects.

Keeping in view buffalo meat as a potential meat source for development of emulsion sausage incorporated with the whey proteins products; the study was conducted with the following objectives of the investigation:

1. To develop emulsion sausage of buffalo meat, incorporating whey protein products namely whey protein concentrate, whey protein isolate and whey protein powder.

2. To optimize the levels of the incorporation for optimum quality.

3. To study the physico-chemical, microbiological and sensory properties of the developed product.

4. To study the shelf life of the product after cooking in casing and further packaging in low density polyethylene.

Keeping in view these objectives of the study, a number of experiments were carried out to evaluate the physic chemical, microbial and sensory attribute and shelf life of the products.

Meat samples collected from the local meat shop in the study were from buffaloes slaughtered according to the traditional halal method at the slaughter house of the municipal corporation, Aligarh. Meat samples from a round portion (biceps fermoris muscle) of 2.5, 3 and 3.5 years aged female carcasses of good finish were obtained from the meat shop within 4 hr. of slaughter. The meat chunks and buffalo fat were packed in combination film packaging and brought to the laboratory within 20 min and kept at 2°C in low temperature cabinet (Yarco, India). The connective tissue portions of the samples were removed. Other non-meat ingredients like spices, salt, condiments, casings and HDPE film were procured from the local market. Whey protein concentrate provided by Mahaan proteins Ltd, New Delhi, India and whey protein powder and isolate provided in local market. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at $2^{0}$ C for 20 hours.

The emulsion sausage (ES) prepared from a comminuted mixture of meat, fat, salt, condiments, spices mixtures and whey protein concentrate, isolate and powder with different levels 1, 2, 3 and 4%. The recipe was; meat 2 kg, fat 400 g, spices mix 32 g, salt 45 g, Condiments 50 g and whey protein with different levels 1, 2, 3, 4%. The buffalo meat was ground on a grinder (PRS Technologies, India) at (11°C temperature, through a 0.95cm plate). The ground meat was transferred to bowl chopper (PRS Technologies, India) for further comminution. It was chopped at slow speed (17 rpm) for two minutes, and then ice cubes (50 g) were added and further comminuted for two minutes. As the mix absorbed the moisture received from molten ice, the other ingredients like fat, salt, spices, condiment and whey protein were added and chopping was further continued for five minutes and the remaining ice addition brought temperature in range of 14-16°C during chopping. Entire mix was filled in the stuffing machine (PRS Technologies, India) and collagen casing (25mm dia) was used for filling sausage. The finished sausage was cooked in sausage cooker (Yarco, India, operated by steam) for 20 min at 110°C temperature. Cooked sausages were exposed to chilled water or chill water was spread over cooked sausage. This operation led to the cracking of casing and finally the sausages were packed in LDPE packageing. The finished sausages were stored at 0°C in an ultra low temperature cabinet for future study.

Samples of raw buffalo meat were analyzed for moisture, protein, fat, ash contents and pH (AOAC, 1990). Initial total plate count and yeast and mold counts of these samples were also assessed (APHA, 1992). The quality of sausages developed was evaluated on the basis of physico-chemical characteristics (pH, moisture content, protein content, fat content, water holding capacity (WHC), ash content, extract releas volume (ERV) and TBA (Thiobarbituric acid) number), microbiological characteristics (total plate count, yeast and mold count, coliform count and *salmonella shigella* count). All the samples were evaluated for direct plate count using serial dilution spread plate technique with nutrient agar medium for total plat count, potato dextrose agar for yeast and mold count, Macconkey agar for coliform count and S.S. agar for salmonella shigella count (APHA, 1992). Sensory attributes (colour, flavor, texture, taste, mouth coaching, palatability and juiciness) of the emulsion sausage samples were evaluated by Hedonic rating test as recommended by Ranganna (1994). Data obtained from experimental observation (n=5), were subjected to analysis of variance (two way ANOVA) (Cochron and Cox, 1992). Linear regression was also determined to study the effect of refrigerated storage (0°C) on physic-chemical, microbial and sensory properties of emulsion sausage incorporated with different levels 1, 2, 3 and 4 % of whey protein products (whey protein concentrate, isolate and whey protein powder). Salient features of research findings were as follows:

- The proximate composition of raw buffalo meat obtained after chemical analysis showed that the raw buffalo meat had 74.2 % moisture content, 1.1 % ash content, 18.2 % protein content and 6.9 % fat content. Carbohydrate was absent in lean meat. The pH value of raw buffalo meat was 6.14 and log TPC/g value was 3.1 while yeast and mold, coliform bacteria and *Salmonella shigella* were absent in the lean meat.

- Moisture contents of emulsion sausage samples incorporated with different levels (1, 2, 3 and 4 %) whey protein concentrate, isolate and whey protein powder were found to be in between 64.40-64.82%, 64.04-64.21% and 64.71-66.12% (on the wet basis) respectively in fresh condition. Different levels of whey protein products slightly increased the moisture content of emulsion sausage samples.
- Refrigerated storage *significantly* ($p<0.05$) *decreased the moisture content* of emulsion sausage samples. It was noted that decreases in moisture contents were due to the evaporation of moisture through the permeable packaging film. The values of $R^2$ were very close to 1 and hence the perfect linear relation was exhibited for consistent reduction in moisture content during refrigerated storage.
- pH values of emulsion sausage samples treated with different levels (1, 2, 3 and 4%) whey protein concentrate, isolate and whey protein powder were found to be in between 6.23–6.37, 6.28-6.37 and 6.31–6.36 respectively in fresh condition. Addition of whey protein products to emulsion sausages made a slight decrease of pH of sausage samples as compared to the control sample.
- During refrigerated storage at 0°C, pH values were found to decrease consistently, and refrigerated storage significantly ($p<0.05$) reduced the pH value of emulsion sausages incorporated with different levels (1, 2, 3, and 4%) whey protein concentrate, isolate and whey protein powder. The values of $R^2$ were near to 1, thus the graph may be approximated to a straight line and linear relation pH and storage period well fits a linear model.
- In fresh condition the ash contents of emulsion sausages of control sample was 2.36% and samples incorporated with different levels whey protein concentrate of 1, 2, 3 and 4% had 2.11, 2.13, 2.15 and 2.17% respectively. The ash contents of emulsion sausage incorporated with whey protein isolate with levels of 1, 2, 3, 4% were found to be 2.29, 2.25, 2.20 and 2.21% respectively, and the ash contents of emulsion sausages samples incorporated with whey protein powder at levels of 1, 2, 3 and 4% had 2.36, 2.18, 2.54 and 2.34% respectively. There was a significant ($p<0.05$) difference between control sample and emulsion sausages incorporated with whey protein concentrate. There were significant ($p<0.05$) decrease in ash content of sausage samples incorporated with whey

protein concentrate, isolate and powder. This were may due to the that mineral of meat polymerized with the mineral of whey protein products.

- Refrigerated storage significantly ($p<0.05$) increased the ash content of emulsion sausages treated with different levels (1. 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder. ANOVA was found that the use of different level of whey protein products (whey protein concentrate, isolate and powder) a significant ($p<0.05$) for ash content of emulsion sausages samples. The increasing nature of ash content with storage time was perfect and the values of $R^2$ was very near to 1, and it indicated that correlation are almost perfect and the graphs may be approximated to a straight line.

- The protein content of control sample of emulsion sausage was found to be 22.58% and sample of sausage with incorporation of whey protein concentrate, isolate and whey protein powder with different levels of 1, 2, 3 and 4% were found to be in range 23.58, 24.10% and 23.38, 24.42% and 24.90, 25.22% respectively. It showed that the whey protein products (whey protein concentrate, isolate and powder) significantly ($p<0.05$) increased the protein contents of emulsion sausage. This result may be explained by the fact that the whey protein products have high protein content.

- Refrigerated storage significantly ($p<0.05$) increased the protein content of emulsion sausages treated with different levels (1, 2, 3 and 4%) whey protein concentrate, isolate and whey protein powder. Perhaps this happened due to the loss of moisture content during the storage period. The values of $R^2$ were near to 1 thus the graph may be approximated to a straight line and linear system well fits between storage period and protein content values.

- Emulsion sausage samples incorporated with different levels of whey protein products (whey protein concentrate, isolate and powder) had significantly ($p<0.05$) lower percentage of fat content as compared to control sample.

- Refrigerated storage significantly ($p<0.05$) increased the fat content of emulsion sausages incorporated with different levels whey protein concentrate, isolate and whey protein powder. Accordingly at the end of $25^{th}$ day of storage, fat content of emulsion sausage incorporated with different levels (1, 2, 3 and 4 %) of whey protein concentrate, isolate and powder were found in the range of 14.61 to

15.44%., 14.12 to 14.69% and 14.12 to 14.69% respectively. The values of $R^2$ for all samples were found to be very near to 1 which shows that correlation were almost perfect and the graphs may be approximated to a straight line.

- In fresh condition TBA number values of emulsion sausage samples treated with whey protein concentrate, isolate and powder were found in range of 0.174-0.191, 0.206–0.230 and 0.196–0.208 respectively. Addition of whey protein products slightly increased the TBA number of emulsion sausage as compared to control sample.

- Refrigerated storage significantly ($p<0.05$) increased the TBA number of all samples. Values of $R^2$ were very close to 1 and the graph may be approximated to a straight line and linear relation well fits between storage period and TBA number values.

- The analysis of variance (ANOVA) shows that the use of different levels of whey protein products significantly ($p<0.05$) increased the extract release volume of sausages sample.

- Refrigerated storage significantly ($p<0.05$) decreased the extract release volume of emulsion sausages incorporated with different levels (1, 2, 3 and 4%) whey protein concentrate, isolate and whey protein powder. The extract release volumes of samples treated with whey protein concentrate, isolate and powder were found in the range of 25.2–27.3, 25.3–27.6 and 25.5–27.3 ml respectively after 25 days of storage. Values of $R^2$ were very close to 1 and hence the perfect linear relation was holding for consistent reduction in extract release volume during refrigerated storage.

- The initial water holding capacity of emulsion sausages treated with different levels (1, 2, 3 and 4% ) of whey protein concentrate, isolate and powder were in range 71.18-71.50%, 72.08-72.89, and 74.60-75.37% respectively. The analysis of variance (ANOVA) showed that the use of different levels of whey protein products significantly ($p<0.05$) increased the water holding capacity of sausages sample as compared to control sample.

- During refrigeration storage it was found that water holding capacity of emulsion sausage samples significantly ($p<0.05$) decreased. Values of $R^2$ were very close to 1 and hence the perfect linear relation was holding for consistent reduction in moisture content during refrigerated storage.

- Total plate count of all samples of emulsion sausages were enumerated in fresh condition and periodically after every 5 days during refrigerated storage. The results of total plate count expressed as log cfu/g. The samples of emulsion sausages incorporated with 1, 2, 3 and 4% of whey protein concentrate isolate and powder have log TPC/g values 3.52, 3.60, 3.64,3.69 and 3.98, 3.85, 3.80 and 3.70, 3.93, 3.92, 3.92 respectively. Different levels of whey protein products significantly ($p<0.05$) increased the total plate counts of emulsion sausages.
- Refrigerated Storage significantly ($p<0.05$) increased total plate count of emulsion sausages incorporated with different levels of whey protein concentrate, isolate and powder. The total plate count was found to be between 7.11- 7.39 log cfu/g, 6.74- 7.23 log cfu/g and 6.76-7.21 log cfu/g on 25th day of storage for samples prepared with different levels (1, 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder respectively. After 25 days of refrigerated storage (0°C) total plate count of all samples were found to be in the safe limit (3.32 - 3.93 log cfu/g). The ANOVA result indicated that the different whey protein products significantly ($p<0.05$) increased total plate count of emulsion sausages. The increasing nature of TPC with storage time was perfect at $R^2=1$, the values of $R^2$ for all samples were found near 1 which shows that correlations are almost perfect and the graphs may be approximated to a straight line.
- Yeast and mold count were not detected in samples till 5 days of refrigerated storage at 0°C. A to few to count was observed at 10th day of storage. However, countable colonies were noted in sausage samples on 25th day of storage in three treatments of whey protein products (whey protein concentrate, isolate and powder). Refrigerated storage significantly ($p<0.05$) increased the yeast and mold count of emulsion sausages. The yeast and mold count was found to be between 3.34-3.86 log cfu/g, 3.45-3.57 log cfu/g and 3.58-3.95 log cfu/g for emulsion sausages incorporated with whey protein concentrate, isolate and powder in varied levels (1, 2, 3 and 4%) respectively. The ANOVA indicated that the different levels of whey protein concentrate isolate and powder incorporated significantly ($p<0.05$) increased yeast and mold count of emulsion sausages. The positive sign in the coefficients of x explains that there was constant increase yeast and mold count during refrigeration storage. Values of $R^2$ were very close

to 1. Thus, the graph may be approximated to a straight line and linear relation well fits between storage period and yeast and mold count.

- Coliform count of emulsion sausages produced with different whey protein product namely whey protein concentrate, isolate and powder were enumerated and it was found that there were no sign of coliform bacteria on plates containing MacConkey agar till 20th days of storage (0°C). However, coliform count were detected in emulsion sausages samples and it was found to be in the range of 2.06-2.34 log cfu/g, 2.15-2.28 log cfu/g and 2.08-2.22 log cfu/g in emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively after 15 days of storage. Refrigerated storage significantly ($p<0.05$) increased the coliform count. In final stage of storage, coliform count was found to be in the range of 2.16-2.54 log cfu/g, 2.30-2.41 and 2.30-2.43. The end of 25th day of refrigerated storage (0°C) coliform count of all sausage samples were found to be in the safe limit.

- *Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C for 25 days.

- The emulsion sausages had bright red colour after cooking. All the fresh emulsion sausages incorporated with whey protein concentrate, isolate and powder at different levels of (1, 2, 3 and 4%) had score values of colour between 8–9. Different levels of whey protein products significantly ($p<0.05$) increased the colour score values of fresh emulsion sausages compare to control sample. The colour score values during storage were found to be decreasing.

- Refrigerated storage significantly ($p<0.05$) decreased the colour score values of emulsion sausage samples. The values of $R^2$ for all samples at four levels of whey protein concentrate, isolate and powder were between 0.9292-0.9498, 0.9363-0.9898 and 0.9429-0.9842 respectively. This shows that relations between colour score and storage period were almost perfect and the graph may be approximated to a straight line.

- In the present study all the emulsion sausages samples incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) had score values of aroma between '8' to '9'. Different levels of whey protein products (whey protein concentrate, isolate and powder) did not significantly ($p<0.05$) affect the aroma of emulsion sausages.

- The interaction between whey protein products and storage period significantly ($p<0.05$) decreased the aroma score values of emulsion sausage samples treated with different levels of whey protein products. During refrigerated storage the score values of aroma of emulsion sausages significantly ($p<0.05$) decreased, and during 25 days of storage it was found in the ranges 6.3-7.0, 5.8-6.2 and 6.1-6.3 for emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively. The values of $R^2$ for all samples at levels 1, 2, 3 and 4% of whey protein concentrate, isolate and powder were between 0.9589–0.9954, which shows relation between aroma of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

- The emulsion sausage samples incorporated with whey protein concentrate at the level of 4% and whey protein isolate at level of 1% and whey powder at the level of 4% received highest score values of texture 8.7, 8.6 and 4% respectively. The lowest score values were for the samples incorporated with whey protein concentrate at the level of 1%, isolate at the level of 4% and whey powder at the level of 1% were 8.1, 8.2 and 8.3 respectively. In fresh condition different levels of whey protein products significantly ($p<0.05$) increased the texture score values of emulsion sausages at the levels of 1 to 4% whey protein concentrate, isolate and powder.

- During the refrigerated storage (0°C) the score values of texture of treated emulsion sausages significantly ($p<0.05$) decreased. The mean score values of texture on 25$^{th}$ day of storage for all samples were between of 5.5-6.6. The $R^2$ for all samples at different levels of whey protein products were between 0.9239–0.9976, which shows relation between texture of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

- All the emulsion sausages samples in fresh condition had the score values of taste between 8.1 to 8.7. It represented condition between liked very much and liked extremely. Different levels of whey protein concentrate, isolate and whey protein powder significantly ($p<0.05$) increased the taste score values in fresh condition of emulsion sausages.

- The interaction between whey protein products (whey protein concentrate, isolate and whey protein powder) and storage period significantly ($p<0.05$) decreased the taste score values of emulsion sausage samples. At the end of storage period (on $25^{th}$ day), there was significant ($p<0.05$) decrease score values taste of different sausages samples treated with whey protein products. The means score values of taste on $25^{th}$ day of storage were between of 6.9-7.5for all samples. Sample treated with whey protein concentrate at the level of 3% had highest scored value (7.5) for the taste among all samples. The values of $R^2$ show relation between taste of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

- All the emulsion sausages in fresh condition had the score values of mouth coating between 7.9 to 8.5. It represented condition between liked very much to like extremely. Different levels of whey protein concentrate, isolate and whey protein powder did not significantly ($p<0.05$) decreased score values of the mouth coating in fresh condition of emulsion sausage samples.

- Refrigerated storage at $0^{\circ}C$ significantly ($p<0.05$) decreased the mouth coating score values of emulsion sausage samples. Sample incorporated with whey protein concentrate at the level of 3 and 4% significantly ($p<0.05$) scored the highest value for the mouth coating among all samples. While lowest score values were given to the sausage samples incorporated with 2 % whey protein powder. The values of $R^2$ for all emulsion sausage samples prepared with whey protein concentrate at the levels (1, 2, 3 and 4%) were in the range of 0.9512-0.9916, for whey protein isolate the range was 0.9434–0.9925 and for whey protein powder the range was 0.949-0.9965.

- All sausage samples treated with whey protein concentrate, isolate and whey protein powder with levels (1, 2, 3 and 4%) have score values of juiciness ranging between 7-9. It represented conditions between like moderately to like extremely. Different levels of whey protein isolate and powder did not significantly ($p<0.05$) increased the juiciness score values of fresh condition of emulsion sausages. But whey protein concentrate significantly ($p<0.05$) increased juiciness score values of the emulsion sausages. It represented conditions between like moderately to like extremely.

- Refrigerated storage significantly ($p<0.05$) decreased the score values of juiciness of emulsion sausage samples treated with different levels of whey protein products. The score values of juiciness all of the samples on 25th day of storage were between of 6.6–7.1 for all samples. The values of $R^2$ for all samples of emulsion sausages incorporated with whey protein products at the levels of 1-4 % were between 0.9325–0.997.

- All the emulsion sausage samples in fresh condition had the score values of palatability between 7.8 to 8.5. It represented condition between liked very much and liked extremely. Different levels of whey protein concentrate, isolate and whey protein powder did not significantly ($p<0.05$) increased score values of palatability of emulsion sausages in fresh condition.

- During refrigerated storage at 0°C the score values of palatability significantly ($p<0.05$) decreased for the emulsion sausage samples treated with different levels (1, 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder. The values of $R^2$ for all sausages samples produced with whey protein products at the levels of 1-4% were in the range of 0.9502-0.999. The values of $R^2$ exhibits that a linear system follows for the relation between palatability score values and storage period, and graph may be approximately represented by a straight line.

- All the emulsion sausage samples treated with whey protein concentrate, isolate and whey protein powder with different levels (1, 2, 3 and 4%) had the score values of overall acceptability between 8 to 9. It represented condition between like very much to like extremely. Different levels of whey protein products significantly ($p<0.05$) increased the overall acceptability score values of fresh condition of emulsion sausages samples.

- Interaction between whey protein products and storage period significantly ($p<0.05$) decreased the overall acceptability score values of emulsion sausage samples. The values of $R^2$ for all samples of emulsion sausage treated with whey protein products with different levels (1, 2, 3 and 4%) were between 0.9161–0.9967, which shows relation between acceptability of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

- The measurements of colour by Hunter Lab of emulsion sausage samples were done in fresh condition and during refrigerated storage while sample was packed in combination film. The numerical value of 'L' in 25 days of storage samples condition found in the range of 18.86-27.77, 16.11-23.39 and 21.35–24.62 for emulsion sausage incorporated with whey protein concentrate, isolate and powder respectively. The sample emulsion sausage incorporated with whey protein concentrate at the end of 25$^{th}$ day storage had 27.77% of maximum lightness as compared to 72.23% darkness. It was brown red colour in appearance. The samples of emulsion sausage incorporated with whey protein isolate at the end of 25th day of refrigerate storage had 23.39% of maximum lightness as compared to 76.61% darkness. The sample of sausage was brown red colour in appearance. The sample of emulsion sausage incorporated with whey protein powder at the end of 25$^{th}$ day of storage had 24.62% of maximum lightness as compared to 75.38% darkness. It was brown red colour in appearance and Hue values were found in the range 3.81- 5.96.

**Following conclusion was drawn from the study**

1. Whey protein products namely whey protein concentrate, isolate and whey protein powder incorporation with buffalo meat emulsion sausage brought considerable improvement in quality characteristics of emulsion sausages samples in fresh condition. It had shown that addition of whey protein products increase the protein content, moisture content, ash content, water holding capacity and extract release volume in fresh emulsion sausages samples. pH values and fat content buffalo meat emulsion sausages decreased with treated of whey protein products.
2. Quality of emulsion sausages samples was evaluated during refrigerated storage (0°C). Refrigerated storage significantly ($p<0.05$) decreased moisture content, pH, water holding capacity and extract release volume of emulsion sausages samples, while the remaining properties (protein content, fat content, ash content and TBA number) significantly ($p<0.05$) increased in duration of storage.
3. In fresh condition different levels of whey protein products (whey protein concentrate, isolate and whey protein powder) significantly ($p<0.05$) increased the total plate counts of emulsion sausages.
4. Refrigerated storage significantly ($p<0.05$) increased total plate count, yeast and mold count and coliform count of emulsion sausages treated with whey

protein products at 0°C. On 25$^{th}$ day of refrigerated storage (0°C) total plate count of all samples were found to be in the safe limit (3.32-3.93 log cfu/g), yeast and mold count was found to be less than 4 log cfu/g and , coliform count was found to be in the range of 2.16-2.54 log cfu/g. *Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C for 25 days.

5. Emulsion sausages treated with whey protein products were acceptable to the panelist. All the fresh emulsion sausages incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) had score values of sensory between 8–9. Refrigerated storage significantly ($p<0.05$) decreased the sensory score values of emulsion sausage samples.

6. Colour measurements of emulsion sausages incorporated with different levels of whey protein products (concentrate, isolate and powder) were also done by Hunter Lab. The measurements of colour of samples were done in fresh condition and during refrigerated storage while sample was packed in combination film. The numerical value of 'L' in 25 days of storage samples condition found in the range of 18.86-27.77, 16.11-23.39 and 21.35–24.62 for emulsion sausage incorporated with whey protein concentrate, isolate and powder respectively. The sample emulsion sausage incorporated with whey protein concentrate at the end of 25$^{th}$ day storage had 27.77% of maximum lightness as compared to 72.23% darkness. It was brown red colour in appearance. Hue values were found in the range 4.78 – 6.74. The samples of emulsion sausage incorporated with whey protein isolate at the end of 25$^{th}$ day of refrigerate storage had 23.39% of maximum lightness as compared to 76.61% darkness. The sample of sausage was brown red colour in appearance. Hue values were found in the range 3.81-5.96. The sample of emulsion sausage incorporated with whey protein powder at the end of 25$^{th}$ day of storage had 24.62% of maximum lightness as compared to 75.38% darkness. It was brown red colour in appearance and Hue values were found in the range 3.81- 5.96.

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# EFFECT OF INCORPORATION OF WHEY PROTEIN PRODUCTS ON QUALITY OF BUFFALO MEAT EMULSION SAUSAGE

THESIS

SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

**Doctor of Philosophy**

IN

POST HARVEST ENGINEERING AND TECHNOLOGY

BY

**ABDOLGHAFOUR BADPA**

UNDER THE SUPERVISION OF

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**2014**

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**Department of Post Harvest Engineering and Technology**
**Faculty of Agricultural Sciences**
**Aligarh Muslim University, Aligarh-202002, UP, India**

# CERTIFICATE

**This to certify that the thesis entitled "Effect of Incorporation of Whey Protein Products on Quality of Buffalo Meat Emulsion Sausage"** submitted to Aligarh Muslim University, Aligarh, for the award of the degree of **Doctor of Philosophy** in **Post Harvest Engineering and Technology** is the original work of **Mr. Abdolghafour Badpa** carried out under my supervision and guidance and no part of this thesis has been submitted for award to any other degree or diploma.

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**Associate Professor**

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# CANDIDATE'S DECLARATION

I, Abdolghafour Badpa Department of Post Harvest Engineering and Technology, certify that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of Dr. Saghir Ahmad at Aligarh Muslim University, Aligarh. The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree.

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This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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**Department:** Post Harvest Engineering and Technology

28-11-14

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This is to certify that Mr. Abdolghafour Badpa, Department of Post Harvest Engineering and Technology has satisfactorily completed the course work/comprehensive examination and pre-submission seminar requirement which is part of his Ph.D. programme.

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# *Dedicated*

*This thesis is dedicated to my wife Helen and my parents who has supported me all the way since the beginning of my research work and has been a great source of motivation and inspiration.*

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**Abdolghafour Badpa**

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| $ | Dollar |
| & | And |
| / | Divide |
| °C | Degree Centigrade |
| °F | Degree Fahrenheit |
| A | Ampere |
| ADP | Adenosine Diphoshate |
| ADPI | American Dairy Products Institute |
| ALP | Alkaline Phosphatise |
| AMP | Adenosine Monophosphate |
| AMU | Aligarh Muslim University |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Analytical Chemists |
| APC | Aerobic Plate Counts |
| APEDA | Agricultural & Processed food product Export Development Authority |
| APHA | American Public Health Association |
| ATP | Adenosine Triphosphate |
| $a_w$ | Water activity |
| BBQ | Barbecue sauce |
| BMI | Body Mass Index |
| BSA | Bovine Serum Albumin |
| CAGR | Compounded Average Growth Rate |
| cfu/g | Colony forming unit per gram |
| CIS | Commonwealth of Independent State |
| Cs | Control Sample |
| CY | Calendar Year |
| DGCI&S | Directorate General of Commercial Intelligence and Staticals |
| DGFT | Directorate General of Foreign Trade |
| DNA | Deoxyribonucleic acid |
| Dr. | Doctor |
| DSP | Disodium Phosphate |
| EC | Emulsifying Capacity |
| EKWE | Ehanolic Kiam Wood Extract |
| EPIC | European Prospective Investigation into Cancer |
| ERV | Extract Release Volume |
| ES | Emulsion Sausage |
| ES | Emulsion Stability |
| EU | European Union |
| FAO | Food and Agriculture Organization |
| FDA | Food and Drug Administration |
| Fig | Figure |
| FSANZ | Food Standards Australia New Zealand |
| G | Gram |
| GMP | Glycomacropeptide |
| GOI | Government of India |
| Hr | Hour |

| | |
|---|---|
| Hz | Hertz |
| ICBF | Irish Cattle Breeding Federation |
| IMP | Inosin Monophosphate |
| Kg | Kilogram |
| LDPE | Low Density Polyethylene |
| LSD | Least Significant Difference |
| Ltd | Limited |
| M | Million |
| M.T | Million Tones |
| mg | Milligram |
| ml | Millilitre |
| MSG | Mono Sodium Glutamate |
| NA | Nutrient Agar |
| Na Cl | Sodium Chloride |
| ND | Not detected |
| NFDM | Non-fat dry milk |
| NIFCS | North South Ireland food consumption |
| Nm | Nanometre |
| NPN | Non protein nitrogen |
| NSS | Normal Saline Solution |
| OECD | Organization for Economic Co-operation and Development |
| PDA | Potato dextrose agar |
| PP | Polyphosphates |
| PPM | Part Per Million |
| $R^2$ | correlation coefficient |
| RDA | Recommended Daily Allowance |
| RH | Relative humidity |
| RNA | Ribonucleic acid |
| rpm | Revolutions per minute |
| Rs | Rupees |
| SA | Sodium ascorbate |
| SAPP | Sodium acid pyrophosphates |
| Sci. | Science |
| SD | Standard Deviation |
| Sec | Second |
| SHMP | Sodium hexameta phosphates |
| Sig | Significant |
| SMP | Skim Milk Powder |
| SMS | Stable Micro System |
| SPP | Sodium Pyrophosphate |
| STPP | Sodium Tripoly Phosphate |
| SW | Meat Swelling |
| TBA | Thiobarbituric acid |
| TBARS | Thiobarbituric acid reactive substance |
| TCA | Tricarboxylic Acid |
| TE | Trade Export |
| TFTC | Too Few to Count |
| TPA | Texture Profile Analysis |

| | |
|---|---|
| TPC | Total Plate Count |
| UAE | United Arab Emirates |
| USDA | United State Department of Agriculture |
| USFDA | United State Food and Drug Administration |
| UTM | Universal Testing Machine |
| V | Volt |
| WBC | Water Binding Capacity |
| WHC | Water Holding Capacity |
| WOF | Warm over flavor |
| WP | Whey Protein |
| WPC | Whey Protein Concentrate |
| WPI | Whey Protein Isolate |
| WPP | Whey Protein Powder |
| WPPs | Whey Protein Products |
| WPs | Whey Proteins |
| WSP | Water Soluble Protein |
| Y & M | Yeast and Mold Count |
| YR | Year |
| β | Beta |
| μmol | Micromole |

# CHAPTER-1

# Introduction

# CHAPTER-1

# INTRODUCTION

Meat and meat product are nutritionally rich, providing a wide range of nutrients, such as protein, fat, minerals and vitamins and constitute an important part of the European diet (Cosgrove *et al.,* 2005). Meat has long been considered a highly desirable and nutritious food, and has become a mass consumer product throughout the world with the highest consumption rates being recorded in industrialized Western countries. Meat is a very versatile culinary product and has become a vital element of both cuisine and culture.

Meat and meat products make an important nutritional contribution to the diet of the people. A significant percentage of the recommended dietary allowances for proteins, vitamins- B, magnesium, iron and zinc are contributed by red meat and poultry (Pearson and Brooks, 1978). Meat is also highly desired food, which fat, protein, minerals and a small proportion of carbohydrate. It is recognized as a highly nutritious food being an excellent source of high quality protein, containing a well balance of the essential amino acids and having high biological value. Meat also contributes a significant percentage of number of other minerals including sodium, potassium, phosphorus, magnesium, calcium, iron and zinc. The nutritional composition (per 100g) of lean red meat is given in Table 1.1.

The world buffalo population is estimated at 185.29 million head, spread in some 42 countries, of which 179.75 million (97%) are in Asia (FAO, 2008). During the last 10 years, the world buffalo population increased by approximately 1.49% annually, by 1.53% in India, 1.45% in Asia and 2.67% in the rest of the world (Antonio and Marco, 2005).

India has a major show in world buffalo population; Pakistan and China are second and third in the world of the buffalo population. India has 105.1 million and they comprise approximately 58 percent of the total world buffalo population, which Pakistan and China have respectively 29.0 million and 23.27 million and India has share 50.34 % of buffalo meat production. (Badpa and Ahmad, 2014a).

The meat production has increased from 764,000 tonnes in 1970-71 to 6.27 million tonnes in 2010. The compounded average growth rate (CAGR) during the last two decades works out to be 4.5%. It is noticed that about 10.6% cattle, 10.6% buffaloes, 24.1% sheep, 58.7% goats, 95.0% pigs and 190.0% chicken are slaughtered each year (Meat Trade News, 2012). Total global meat production per person has steadily increased from 0.13 lbs per day in 1961 to 0.29 lbs per day in 2009, a 120 percent increase over the last half century. Fig 1.1 shows world meat production per person 1961-2009 (Guyenet, 2012). The world total meat production during 2012 was 301.8 MT buffalo meat production was 3.59 MT.

The present production of meat of India is estimated 9.29 million tonnes (FAO, 2011), standing fourth in rank in world's meat production. The contribution of meat from buffalo is about 17.0%, while cattle 16.0%, sheep 3.0%, goat 6.0%, pig 6.4% and poultry 51.0% (Ranjhan, 2013). Looking into the carcass weight per animal in the country, which is much below the world average in cattle and where as reaching to the world average in buffalo, sheep, and goat. About 33% of meat production in the country is from cattle and buffalo; about 6% from goat whereas about 3% is produced from sheep. India share 3.08% of the total meat production. The table 1.2 shows trends in livestock and meat production in India-2011.

**Table 1.1:** Nutrient compositions (per 100g) of lean red meat

| | Beef | Veal | Lamb | Mutton |
|---|---|---|---|---|
| Moisture (g) | 73.1 | 74.8 | 72.9 | 73.2 |
| Protein (g) | 23.2 | 24.8 | 21.9 | 21.5 |
| Fat (g) | 2.8 | 1.5 | 4.7 | 4.0 |
| Energy (KJ) | 498 | 477 | 546 | 514 |
| Cholesterol (mg) | 50 | 51 | 66 | 66 |
| Thiamin (mg) | 0.04 | 0.06 | 0.12 | 0.16 |
| Riboflavin (mg) | 0.18 | 0.20 | 0.23 | 0.25 |
| Niacin (mg) | 5.0 | 16.0 | 5.2 | 8.0 |
| Vitamin B6 (mg) | 0.52 | 0.8 | 0.10 | 0.8 |
| Vitamin B12 (μg) | 2.5 | 1.6 | 0.96 | 2.8 |
| Pantothenic acid (mg) | 0.35 | 1.50 | 0.74 | 1.33 |
| Vitamin A (μg) | <5 | <5 | 8.6 | 7.8 |
| Beta-carotene (μg) | 10 | <5 | <5 | <5 |
| Alpha-tocopherol (mg) | 0.63 | 0.50 | 0.44 | 0.20 |
| Sodium (mg) | 51 | 51 | 69 | 71 |
| Potassium (mg) | 363 | 362 | 44 | 365 |
| Calcium (mg) | 4.5 | 6.5 | 7.2 | 6.6 |
| Iron (mg) | 1.8 | 1.1 | 2.0 | 3.3 |
| Zinc (mg) | 4.6 | 4.2 | 4.5 | 3.9 |
| Magnesium (mg) | 25 | 26 | 28 | 28 |
| Phosphorus (mg) | 215 | 260 | 194 | 290 |
| Copper (mg) | 0.12 | 0.08 | 0.12 | 0.22 |
| Selenium (μg) | 17 | <10 | 14 | <10 |

(Source- Williams, 2007)

The increase in meat production gives rise to various problems in handling, processing, preservation and storage, marketing and distribution till it reaches to the consumer table. These problems can be solved through research and development and the person involved in the meat industry need significant training to improve their traditional skill. The food animals generally used for production of meat comprise of cattle, buffalo, sheep, goat and poultry (Ahmad, 2005).

**Table1.2:** Trend in Livestock Production and Meat Production in India-2011

| Livestock species | Population (in M) | Animal slaughtered (M) | Percent slaughtered (%) | Carcass weight (kg) | Meat prod-uction (M.T) | Share in total meat produc-tion (%) |
|---|---|---|---|---|---|---|
| Cattle | 195.0 | 14.2 | 7.28 | 103 | 1.49 | 16.0 |
| Buffalo | 105.0 | 10.3 | 9.80 | 138 | 1.58 | 17.0 |
| Sheep | 57.0 | 19.2 | 35.68 | 12 | 0.25 | 3.0 |
| Goat | 140.0 | 47.0 | 33.57 | 10 | 0.57 | 6.0 |
| Pigs | 18.0 | 16.0 | 88.90 | 31 | 0.60 | 6.4 |
| Poultry | 1049.0 | 604.0 | 57.57 | 0.8 | 4.80 | 51.0 |
| Total | | | | | 9.29 | 100 |

Source: Department of Animal Husbandry & Dairying, 2012, FAO-2012

Meat consumption is based largely on availability, price and tradition. Meat production is a very complex operation depending not only on demand (which is usually based on price and income) but also on many social and economic influences such as official policy, price support mechanisms, and interrelations such as the interaction between beef and milk production, the availability of animal feedstuffs and competition for food between man and animals.

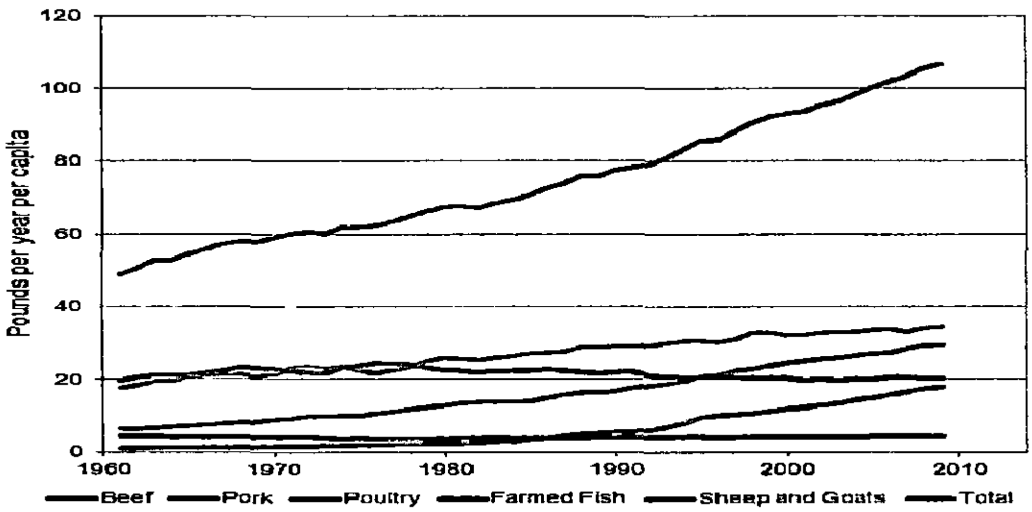



**Fig.1.1**: World meat production per person

Many factors such as wealth, volume of livestock production and socioeconomic status of consumers could explain the higher consumption pattern of meat by western population (Mann, 2000; Speedy, 2003). Other factors influencing meat consumption include sex, age, religion, body mass index (BMI) and total energy intake as reported by the European prospective investigation into cancer and nutrition (EPIC) cohort (Linseisen *et al.*, 2002). In the UK and Ireland men and women's average daily intakes of total meat are 108g, 72g, and168g, 107g respectively (Linseisen *et al.*, 2002; Cosgrove *et al.*, 2005). Table 1.3 shows mean daily intakes of total red and processed meats among consumer of Ireland, UK and several European countries measured by North South Ireland Food Consumption (NSIFCS) and European Prospective Investigation in to Cancer (EPIC) respectively.

Merco Press (2012) reported that the last year per capita consumption of the meat in the world was 42.3 kg, while it was 42.5 kg in the previous year. In a wealthy nations meat consumption is on average 78.9 kg per person, versus 32.3 kg in developing nations. Interestingly, accompanying the slight decrease in consumption was a slight rise in meat production. In 2011, 297 million tonnes of meats were produced globally, while showed an increase of 0.8 % over 2010 levels. Meat production this year (2012) on track to hit 302 million tonnes. Approximately 70% of the country's population consumes meat.

India's per capita buffalo meat consumption is estimated at approximately two kg per year. Meat consumption increases will likely occur primarily in the poultry segment. CY 2013 buffalo meat consumption in India is forecast at 2 million tonnes (on a carcass weight equivalent basis), CY 2012 buffalo meat consumption is estimated at 1.98 million tonnes, and CY 2011 buffalo meat consumption is kept unchanged at 1.95 million tonnes. Note that despite an increase in overall consumption, per capita consumption may not arise due to India's growing population.

**Table 1.3:** Daily intakes of total red and processed meats among consumer of Ireland, UK and several European countries

| Country | Total[a] | | Red[b] | | Processed meat[c] | |
|---|---|---|---|---|---|---|
| | Men | Women | Men | Women | Men | Women |
| UK[d] | 108.1 | 72.3 | 4 | 24.6 | 38.4 | 22.3 |
| Ireland[e] | 167.9 | 106.6 | 63.9 | 37.5 | 30.9 | 19.9 |
| Greece[d] | 78.8 | 47.1 | 45.3 | 25.5 | 10 | 5.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Spain[d] | 170.4 | 99.2 | 74 | 37.8 | 52.8 | 29.6 |
| Germany[d] | 154.6 | 84.3 | 52.2 | 28.6 | 83.2 | 40.9 |
| Italy[d] | 140.1 | 86.1 | 57.8 | 40.8 | 33.5 | 19.6 |
| Denmark[d] | 141.1 | 88.3 | 69.6 | 44.1 | 51.9 | 25.3 |
| Netherlands[d] | 155.6 | 92.7 | 63.8 | 41.1 | 72.4 | 37.9 |

a: Total meat (pork, beef, veal, lamb, mutton, poultry, game, rabbit, hours, goat, offal, and processed)
b: Red meat (beef, veal, pork and lamb)
c: Processed meat (ham., bacon, P. M. cuts, minced meat and sausage)
d: Source Linsseisen *et al.*,2002
e: Source Cosgrove *et al.*,2005
Source:McAfee *et al.*, 2010

India is a net exporter of buffalo meat (deboned frozen buffalo meat). In the last two years, exports have grown to record levels, making India the fourth country in the world to export more than 1 million tonnes of bovine meat annually. India's growing exports are the result of its low cost of production (relative to international competitors). Production costs are low due to herd growth of strong dairy demand and new incentives from slaughter facilities to salvage previously underutilized animals. As a result, CY 2013 buffalo meat exports are forecast at 2.15 million tonnes (on a carcass weight equivalent basis), around 30 percent over 1.66 million tonnes in CY 2012. CY 2011 buffalo meat exports are also revised up to a record 1.29 million tonnes, based on trade data. Year-on-year export growth for 2011 is 41 percent, while 2012 growth is 28 percent. Given its tremendous export growth, India is likely to become the world's largest beef (buffalo meat) exporter by 2013, if not sooner. Import of beef from all sources is restricted and as such imports are set at nil. Table 1.4, 1.5 and Fig 1.2 show the world and India's meat potential.

**Table 1.4**: Buffalo Meat Exports from India to Key Market

| | 2011-12 | | 2012-13 | | 2013-14 | |
|---|---|---|---|---|---|---|
| Country | Qty (MT) | Value (Rs lacs) | Qty (MT) | Value (Rs lacs) | Qty (MT) | Value (Rs lacs) |
| Vietnam Social Republic | 2,72,965.81 | 4,04,741.73 | 3,30,108.73 | 5,12,529.93 | 5,24,173.90 | 10,97,157.57 |
| Malaysia | 99,704.82 | 1,40,412.71 | 1,15,222.87 | 1,94,370.48 | 1,21,741.00 | 2,35,642.21 |
| Egypt Arab Republic | 70,173.01 | 1,14,316.41 | 71,193.06 | 1,25,571.14 | 1,07,821.87 | 2,03,330.36 |
| Thailand | 29,708.69 | 43,772.24 | 87,049.61 | 1,44,662.64 | 1,74,018.92 | 1,78,929.45 |
| Saudi Arabia | 65,542.57 | 94,694.69 | 69,570.21 | 1,17,376.87 | 74,598.96 | 1,49,354.37 |
| Jordan | 68,398.06 | 87,781.99 | 49,862.25 | 75,133.68 | 51,181.26 | 91,069.61 |
| Algeria | 43,098.73 | 57,750.44 | 41,717.00 | 63,905.99 | 48,840.00 | 84,968.37 |
| United Arab | 40,338.25 | 49,420.33 | 43,651.70 | 66,599.98 | 42,793.54 | 78,467.89 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Emirates | | | | | | |
| Philippines | 45,476.72 | 54,724.01 | 43,682.18 | 56,982.78 | 45,327.86 | 72,325.94 |
| Iran | 33,019.93 | 43,659.39 | 45,289.77 | 69,612.40 | 37,843.00 | 71,491.59 |
| Angola | 22,152.78 | 28,396.15 | 30,853.05 | 46,013.16 | 35,489.00 | 60,483.45 |
| Iraq | 22,528.09 | 28,410.54 | 24,738.16 | 35,298.87 | 29,992.00 | 52,677.03 |
| Kuwait | 21,089.09 | 28,354.21 | 18,652.26 | 28,675.42 | 20,617.99 | 37,482.81 |
| Oman | 13,968.82 | 19,300.31 | 13,105.13 | 21,535.16 | 13,579.48 | 26,167.38 |
| Congo P Republic | 12,911.77 | 15,817.40 | 18,457.40 | 25,731.26 | 15,229.00 | 24,489.55 |
| Azerbaijan | 4,879.00 | 7,471.75 | 8,204.11 | 13,884.42 | 8,927.00 | 18,596.58 |
| Gabon | 8,087.40 | 9,049.81 | 6,073.00 | 8,221.35 | 9,238.00 | 14,607.08 |
| Lebanon | 7,991.95 | 11,097.78 | 5,523.00 | 8,714.30 | 7,204.00 | 12,963.11 |
| Turkmenistan | 1,285.03 | 1,350.85 | 573.00 | 903.15 | 7,838.00 | 11,359.75 |
| Qatar | 7,234.40 | 11,225.06 | 5,659.62 | 8,781.50 | 5,991.93 | 11,313.69 |
| Syria | 16,763.48 | 22,836.73 | 8,257.89 | 13,392.16 | 5,964.00 | 11,024.49 |
| Laos | 338.00 | 528.66 | 3,859.00 | 6,613.02 | 5,371.00 | 10,510.11 |
| Georgia | 15,451.02 | 20,876.73 | 10,081.84 | 15,632.32 | 4,511.00 | 8,553.96 |
| Congo D. Republic | 0.00 | 0.00 | 1,822.00 | 2,717.41 | 4,602.60 | 7,531.84 |
| Mauritius | 3,417.00 | 5,386.00 | 3,259.22 | 6,034.43 | 3,150.30 | 7,037.31 |
| Tajikistan | 1,677.47 | 2,276.84 | 2,416.20 | 3,621.05 | 3,986.50 | 6,767.32 |
| Senegal | 4,697.14 | 5,766.11 | 3,213.37 | 4,346.74 | 3,804.00 | 6,586.97 |
| Bahrain | 2,730.52 | 4,245.98 | 2,235.51 | 3,726.78 | 3,203.83 | 6,165.22 |
| Armenia | 4,828.78 | 6,564.81 | 3,812.00 | 6,523.39 | 2,570.00 | 5,555.66 |
| Uzbekistan | 1,348.00 | 1,813.75 | 2,479.00 | 3,891.95 | 2,456.00 | 4,348.72 |
| Equtl Guinea | 3,632.00 | 4,098.58 | 1,374.00 | 1,919.56 | 2,584.00 | 4,243.14 |
| Brunei | 1,776.00 | 2,701.55 | 2,100.60 | 3,942.79 | 1,802.00 | 3,963.30 |
| Yemen Republc | 1,851.73 | 2,106.03 | 2,269.66 | 3,134.42 | 2,447.00 | 3,556.04 |
| Hong Kong | 1,539.00 | 2,556.86 | 7,028.36 | 13,525.13 | 1,342.00 | 3,195.86 |
| Pakistan | 4,230.49 | 2,522.12 | 3,625.45 | 2,128.39 | 4,421.37 | 2,977.62 |
| Ghana | 4,859.10 | 4,256.30 | 2,513.00 | 1,990.41 | 2,138.00 | 2,843.12 |
| Taiwan | 0.00 | 0.00 | 0.00 | 0.00 | 1,172.00 | 2,658.10 |
| Comoros | 1,706.46 | 1,944.94 | 2,346.20 | 3,179.82 | 1,674.38 | 2,450.38 |
| Maldives | 891.89 | 1,348.98 | 871.00 | 1,702.92 | 899.00 | 1,820.84 |
| Afghanistan | 1,264.03 | 1,326.91 | 905.40 | 966.97 | 1,467.60 | 1,681.09 |
| Liberia | 2,229.20 | 1,344.69 | 1,876.54 | 1,227.43 | 2,441.00 | 1,638.28 |
| Sierra Leone | 1,141.00 | 1,001.12 | 1,812.43 | 1,628.90 | 1,148.00 | 1,249.90 |
| Cote D Ivoire | 1,786.40 | 1,635.51 | 1,537.47 | 1,605.96 | 980.00 | 1,003.28 |
| Benin | 477.03 | 543.37 | 812.00 | 1,045.34 | 618.00 | 971.56 |
| Namibia | 1,319.00 | 1,542.13 | 476.00 | 736.61 | 476.00 | 901.42 |
| Turkey | 1,168.35 | 1,521.38 | 404.50 | 508.54 | 513.00 | 875.72 |
| Libya | 0.00 | 0.00 | 252.00 | 441.48 | 336.00 | 572.74 |
| Kazakhstan | 354.00 | 473.04 | 0.00 | 0.00 | 225.00 | 527.37 |
| Seychelles | 292.95 | 367.40 | 339.00 | 535.76 | 227.00 | 400.74 |
| Tunisia | 0.00 | 0.00 | 126.00 | 213.42 | 165.00 | 301.49 |
| Singapore | 131.00 | 114.81 | 574.99 | 942.23 | 154.00 | 286.84 |
| Moldova | 0.00 | 0.00 | 364.00 | 524.15 | 140.00 | 240.25 |
| Gambia | 140.00 | 190.64 | 144.00 | 196.13 | 72.00 | 120.33 |
| Korea Republic | 0.00 | 0.00 | 0.23 | 0.11 | 48.00 | 101.35 |
| Guinea | 145.00 | 118.11 | 16.00 | 28.60 | 112.00 | 96.29 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Venezuela | 0.00 | 0.00 | 0.00 | 0.00 | 28.00 | 59.70 |
| Kyrghyzstan | 562.00 | 809.01 | 56.00 | 107.81 | 28.00 | 48.10 |
| Cameroon | 50.00 | 71.24 | 0.00 | 0.00 | 29.00 | 32.04 |
| Nepal | 1.75 | 5.10 | 17.40 | 26.75 | 5.30 | 5.25 |
| Sri Lanka | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.05 |
| Albania | 60.00 | 76.12 | 42.56 | 202.20 | 0.00 | 0.00 |
| Bangladesh | 0.30 | 0.58 | 0.00 | 0.00 | 0.00 | 0.00 |
| Bhutan | 0.00 | 0.00 | 24.12 | 29.00 | 0.00 | 0.00 |
| Bulgaria | 5.00 | 22.90 | 0.00 | 0.00 | 0.00 | 0.00 |
| Canada | 2,426.92 | 2,704.17 | 708.27 | 931.25 | 0.00 | 0.00 |
| Chad | 0.00 | 0.00 | 820.23 | 1,084.29 | 0.00 | 0.00 |
| China P Rp | 1,778.00 | 2,904.22 | 1,744.97 | 2,752.08 | 0.00 | 0.00 |
| Denmark | 29.00 | 32.28 | 0.00 | 0.00 | 0.00 | 0.00 |
| El Salvador | 0.00 | 0.00 | 1.36 | 3.77 | 0.00 | 0.00 |
| France | 0.00 | 0.00 | 0.10 | 0.08 | 0.00 | 0.00 |
| Germany | 0.00 | 0.00 | 7.50 | 14.51 | 0.00 | 0.00 |
| Indonesia | 0.00 | 0.00 | 812.00 | 1,320.55 | 0.00 | 0.00 |
| Italy | 28.00 | 40.74 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mexico | 0.00 | 0.00 | 56.00 | 73.44 | 0.00 | 0.00 |
| Mozambique | 0.00 | 0.00 | 55.00 | 88.03 | 0.00 | 0.00 |
| Myanmar | 7,751.44 | 12,725.91 | 622.51 | 1,334.31 | 0.00 | 0.00 |
| Netherland | 7.30 | 16.84 | 30.50 | 19.65 | 0.00 | 0.00 |
| Romania | 0.00 | 0.00 | 28.00 | 35.51 | 0.00 | 0.00 |
| South Africa | 821.80 | 974.61 | 27.00 | 36.09 | 0.00 | 0.00 |
| Spain | 90.00 | 100.72 | 0.00 | 0.00 | 0.00 | 0.00 |
| Sudan | 0.00 | 0.00 | 28.00 | 46.58 | 0.00 | 0.00 |
| Tanzania Republic | 266.00 | 331.69 | 28.00 | 42.37 | 0.00 | 0.00 |
| United Kingdom | 0.00 | 0.00 | 1.34 | 3.82 | 0.00 | 0.00 |
| United States | 0.00 | 0.00 | 2.40 | 7.58 | 0.00 | 0.00 |
| Zimbabwe | 0.00 | 0.00 | 2.00 | 0.80 | 0.00 | 0.00 |
| Total | 9,86,618.47 | 13,74,574.01 | 11,07,506.23 | 17,41,289.27 | 14,49,758.64 | 26,45,781.58 |

**Source:** DGCIS Annual Export Statistics as on APEDA website (2014)

**Table 1.5**: Buffalo Meat Export from India

| Year | Total meat production | Total buffalo meat production | Total deboned buffalo meat exports* | Percentage* Export of Buffalo Meat Product |
|---|---|---|---|---|
| | (Tonnes*) | (Tonnes) | (Tonnes) | (Percent) |
| 2001-02 | 4,425,000 | 1,421,000 | 240,989 | 17 |
| 2002-03 | 5,622,000 | 1,428,000 | 295,456 | 21 |
| 2003-04 | 5,898,000 | 1,433,000 | 338,940 | 23.40 |
| 2004-05 | 5,922,000 | 1,471,000 | 302,280 | 20 |
| 2005-06 | 6,212,000 | 1,582,000 | 459,937 | 29 |
| 2006-07 | 6,251,000 | 1,621,210 | 494,111 | 30 |
| 2007-08 | 6,302,000 | 1,632,170 | 482,925 | 29 |
| 2008-09 | 6,322,000 | 1,633,500 | 483,737 | 29.1 |
| 2009-10 | 6,524,000 | 1,645,290 | 513,668 | 31.2 |
| 2010-11 | 8,285,000 | 1,825,450 | 724,273 | 38.8 |
| 2011-12 | 9,290,000 | 1,835,920 | 972,863 | 68 |

Source: APEDA, 2012; DGFT (2012); FAO STAT-2012
*Include Poultry Meat

As a price-based competitor, India has seen export increases in the previous two years to Middle Eastern, African and Southeast Asian countries. The export price of buffalo meat has increased in India Rs 47.74 to Rs 142.50 from 2000-01to 2011-12 Directorate General of Foreign Trade (DGFT, 2011-12). The growth price approximately Rs 30 per year in last 2 years.

Buffalo meat can be very well used for production of sausage, a ready to eat and serve product. Sausage is a food that is prepared from comminuted and seasoned meat and is usually formed into a symmetrical shape. The word sausage comes from the Middle English sausige, which came from sal, Latin for salt. In France they are sausissons and in Germany, Wurst.In practice for over a millennia sausage-making was originally a method used to preserve meats, especially lesser cuts (Huda *et al.*, 2012; Marianski and Marianski, 2008).

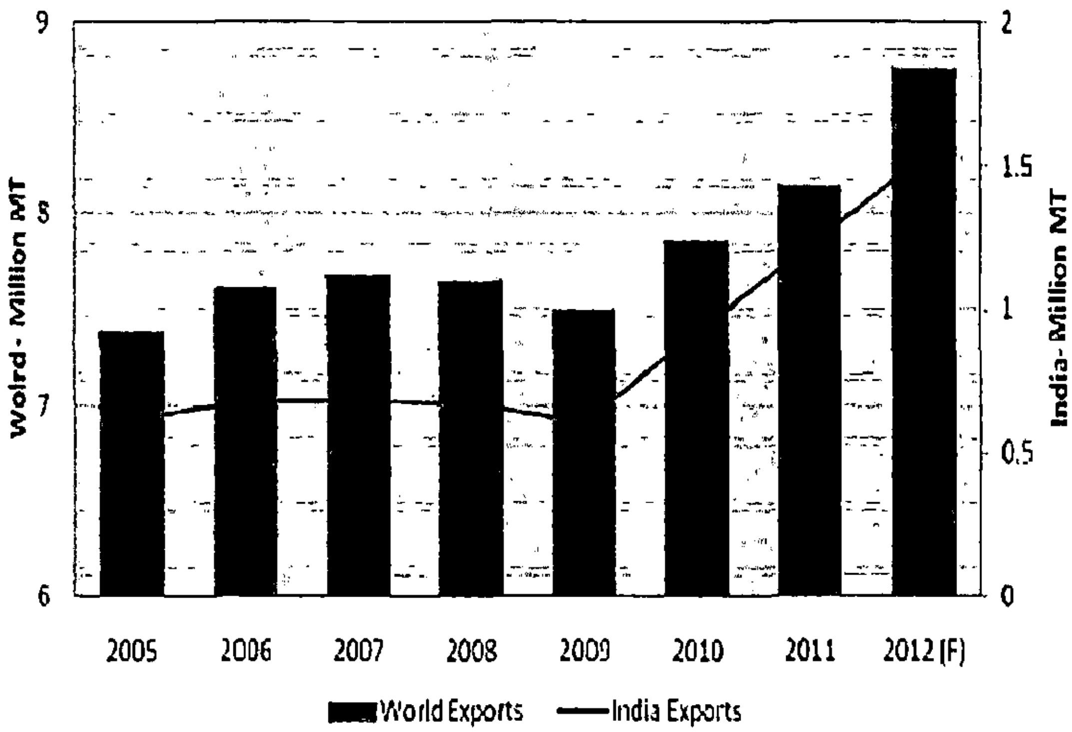



**Fig.1.2**: World and India's meat export (USDA, 2012)

Sausage making had developed over a number of countries, beginning simple process of salting and drying meat. This was done to preserve fresh meat that could not be consumed immediately. The typical flavors, texture, and shape of the many sausages known today, such a frankfurter, braunschweiger, and salami were named due to the geographical location of their origin.

Some sausage is smoked after preparation and cooked before consumption. Fresh sausages are uncured, comminuted, seasoned and usually stuffed into casing and cooked before serving like bratwurst. Cooked sausage are cured/uncured meats, comminuted, seasoned, stuffed into casing, cooked and sometimes smoked usually and served cold e.g. Liver sausage, braunscweiger and liver cheese. Cooked smoked sausages are prepared from cured meats, comminuted, seasoned and stuffed in to casing, smoked and fully cooked and do not require further cooking before serving like Frankfurters and cotto Salami. Uncooked smoked sausages are prepared from fresh meat cured or uncured, stuffed, smoked but not cooked. It must be fully cooked before serving e.g. Metwurst and smoked country sausages.

Sausage may be classified in any number of ways, for instance by the type of meat and other ingredients they contain or by their consistency. The most popular classification is probably according to the processing method. Table 1.6 and flow chart 1.3 have been shows of different types of sausage. There are four main categories of sausages: fresh sausage, cooked sausage, cooked and smoked sausage, uncooked and smoked sausage, dry sausage and specialty meats. The sausages listed here are basically ground meat, seasoned and flavored, with added fat, stuffed into casings. Bulk sausage flavors, ground meat, usually pork, which is cooked like ground beef, or formed into patties.

The emulsion sausages, which that have been finely comminuted to the consistency of a fine paste. Hot dog, Frankfurter, Mortadella, Bologna, liver sausage is typical examples. In cases, they cook with moist heat (steamed or in hot water). Emulsified sausages can be divided in two groups: 1). High quality products made at home such as Austrian wiener or Polish Serdelki which are made from high quality meats and without chemicals. Beef, veal and pork are the meats commonly used. Beef Frankfurter contains beef only. High quality products contain enough lean meat to absorb the necessary water without help from water retention agents. 2). Commercial products made from all types of meat trimmings (pork, beef, chicken, turkey), including machine separated meat. Chicken hot dogs, turkey hot dogs and all possible combinations can be found in a supermarket. A large number of chemicals, water binding agents, fats and water are added during manufacturing to compensate for lower meat grades.

**Table 1.6**: Different Types of Sausage

| SAUSAGE | TYPE | INGREDIENTS | COOKING METHOD |
|---|---|---|---|
| Polish Sausage | Fresh | Pork, beef, garlic, thyme or marjoram, pork fat, pepper | Steam, Fry, Grill, Bake to 155°F |
| Kielbasa | Fresh, Smoked | Beef, pork, garlic, pork or beef fat, mustard | Steam, Fry, Grill, Bake to 155°F |
| Bratwurst | Fresh, sometimes smoked and cooked | Pork or beef, veal, dry milk, onion, garlic, coriander, caraway, nutmeg | Steam, Fry, Grill, Bake to 155°F |
| Salami | Dry, Cured | Highly seasoned: garlic, salt, pepper, sugar | Ready to eat |
| Sweet or Hot Italian | Fresh | Sweet: garlic, sugar, anise, and fennel<br>Hot: paprika, chile peppers, onion, garlic, fennel, parsley | Steam, Fry, Grill, Bake to 155°F |
| Cervelat or Summer Sausage | Cured, Smoked, Semi-Dry | Pork, beef, garlic, mustard, mild spices | Ready to eat |
| Andouille | Smoked | Pork, salt, very spicy, sugar, paprika, red pepper, garlic, sage | Ready to eat |
| Boudin Blanc | Fresh, delicate | Pork, fat, eggs, cream, bread crumbs, seasonings | Gently sauté |
| Braunschweiger | Precooked, smoked | Smoked liver, eggs, milk | Ready to eat; spreadable |
| Boudin Noir | Precooked | Pig's blood, suet, bread crumbs | Ready to eat; better sautéed |
| Knackwurst | Precooked, Smoked | Beef, pork, lots of garlic, cumin | Ready to eat |
| Linguica | Cured, Smoked | Pork butt, lots of garlic, cumin, cinnamon, vinegar | Usually ready to eat |

| | | | |
|---|---|---|---|
| Pepperoni | Air-dried | Pork, beef, lots of black and red pepper | Usually ready to eat |
| Chorizo | Dry, Smoked | Pork, cilantro, paprika, garlic, chili powder, very spicy | Usually ready to eat |
| Mortadella | Semi-dry, Smoked | Cubes of pork fat, pork, beef, peppercorns, garlic, anise | Steam, Fry, Grill, Bake to 155°F |
| Hot Dogs | Cooked, Smoked, Cured | Cured beef and pork, garlic, salt, sugar, mustard, pepper | Ready to eat |
| Bockwurst | Fresh | Veal, pork, milk, chives, eggs | Steam, Saute, Bake to 155 °F |
| Bologna | Cooked, Smoked | Cured beef and pork, garlic, salt | Ready to eat |

**Sources:** Larsen (2013)

Emulsion sausage will be successful if the enough lean meat has been selected and enough myosin has been extracted. The lean meat is the main source of myosin. The more myosin extracted, the thicker and stronger protein coat develops around particles of fat. In cause of myosin depends on how vigorous the cutting process was and how much salt (and phosphates) was added.

Whey proteins are by-products of the chesses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Now a day, great efforts are being made to find new uses for whey proteins, e.g. production of edible film (Anker *et al.,* 1998). Whey protein improves emulsion stability, provide better color properties and result in lower chewiness and elasticity. (Yetmin *et al.*, 2001).

Whey protein is a high quality, complete protein with all the essential amino acids. Whey protein is richest source of naturally occurring branched chain amino acids (leucine, isoleucine and valine). These are important for active individuals, exercise and professional athletes. Adding whey protein to the diet is great ways to jump start a weight loss program. Whey protein is a key ingredient in numerous weight loss and meal replacement products.

It is estimated that the world production of whey is about 104 billion kg per year with the USA producing about 30 billion kg per year (Saddoud *et al.*, 2007; Cheryan, 1998). However, about 30 to 47% of the total amount of whey available worldwide is not being used (Hutchinson *et al.*, 2003; Saddoud *et al.*, 2007).

Whey protein consists of a number of individual protein components. The two most abundant proteins are β-Lac (50-55%) and α-Lac (20-25%). β-Lac has a molar mass of 18.3 kDa and diameter of about 2 mm. The isoelectric point is 5.1 and the denaturation temperature is 78 °C. β-Lac is largely responsible for solubility, gelation, foaming, emulsification, and flavor binding of whey protein. Because β-Lac is the most abundant protein in whey, it has been suggested to be one of the main determinants of the properties of whey protein gels (Van Vliet *et al.*, 2004).

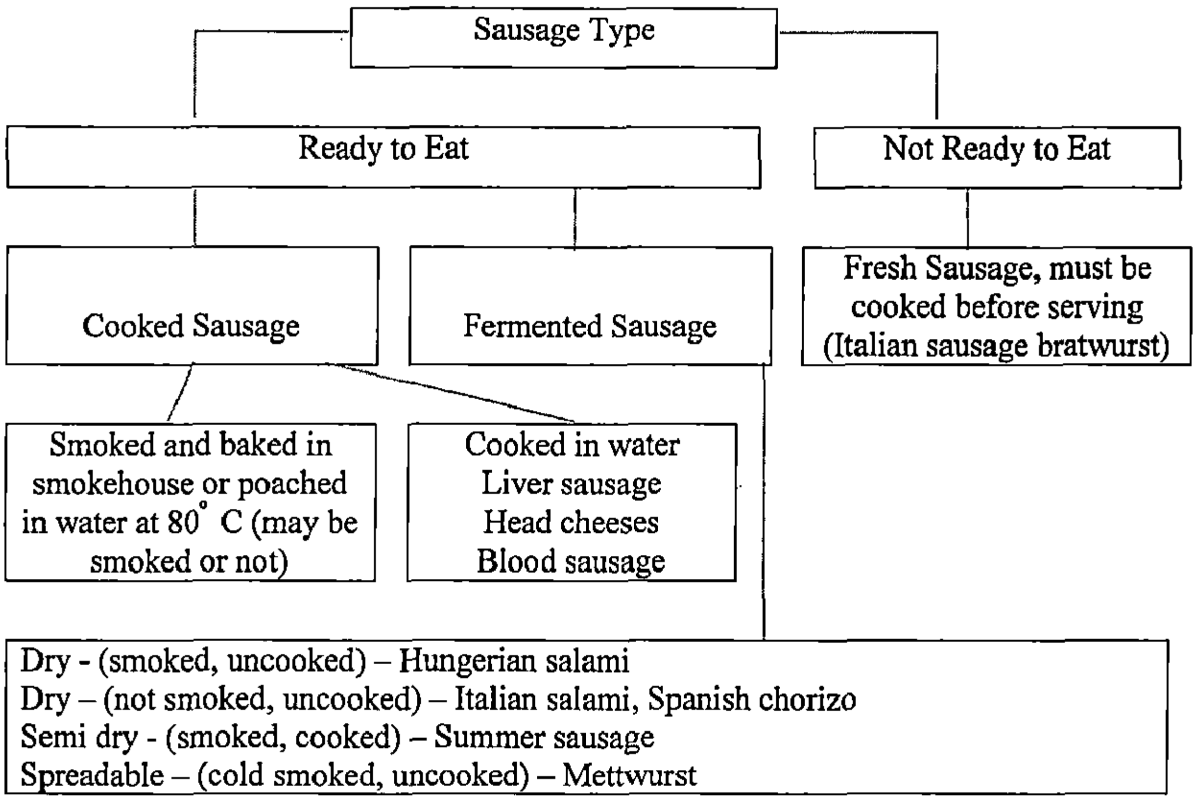



**Fig. 1.3**: Flow chart of different type of sausage (Marianski, 2011)

Emulsion sausage incorporated with whey protein products, can be prepared from buffalo meat using other ingredient salt, spices, condiment, whey protein (isolate, concentrate, whey powder), fat and animal fat. The product will have a pleasant taste, excellent flavor and increased juiciness. Also product contains bioactive ingredients like immuglobulins and lactoferrin, which help to support the immune system. Emulsion sausage incorporated with whey protein products will have rich abundance of branched chain amino acids and its quick absorption rate. These are important to help repair and rebuild muscles. Emulsion sausage treated with whey protein products have fresh, natural taste and do not contain isoflavones and any other component with potential hormonal effects.

There are chances of contamination of meat and meat products during slaughtering, handling and processing. The person, equipment and machineries are potential source of microbiological penetration in the developed products. The most important part of the production of meat and meat products is to make the system hazard free. The industry environment must be hygienic and to ensure the safety and security, hazard Analysis Critical Control Point (HACCP) system should be followed.

Sausages are packed in natural and artificial casing, which provide size and shape. Casing serves as processing mold, as containers during handling and shipping and merchandising it for display. The sausages are packed or stuffed in natural or artificial casing. The natural casing is preserved in salt solution. The artificial is of different type, i.e. cellulosic. Non edible collagen and plastic are to be peeled before consumption. The Casing must be sufficiently strong to contain the meat mass, but have shrink and stretch characteristics that allow contraction and expansion of the meat mass during processing and storage. The casing must withstand the forces produced during stuffing, linking, and closure (Ahmad, 2005).

Keeping in view buffalo meat as a potential meat source for development of emulsion sausage incorporated with the whey protein products; the study was conducted with the following objectives of the investigation:

1. To develop emulsion sausage of buffalo meat, incorporating whey protein products namely whey protein concentrate, whey protein isolate and whey protein powder.
2. To optimize the levels of the incorporation for optimum quality.
3. To study the physico-chemical, microbiological and sensory properties of the developed product.
4. To study the shelf life of the product after cooking in casing and further packaging in low density polyethylene.

# CHAPTER-2

# Literature Review

# CHAPTER-2

# LITERATURE REVIEW

The Food Standards Australia New Zealand (FSANZ, 2002) Food Standard Code defines meat as 'the whole or part of the carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep, slaughtered other than in a wild state, but does not include eggs.

Meat continues to be an important food group in the diet for many consumers, particularly in the developed world (Delgado, 2003; Rosegrant *et al.,* 1999; Speedy, 2003). Total meat can break down into Red meat (beef, lamb, veal and pork), White meat (chicken, game and turkey) and Processed meat (cured and smoked meat, Ham, bacon, sausage, hamburgers, salami and tinned meat) (Linseisen *et al.,* 2002).

## 2.1 Nutritional role of meat in the human diet

Meat has traditionally been considered an essential component of the human diet to ensure optimal growth and development. With a limited range of foods available in societies throughout history, meat was important as a concentrate source of a wide range of nutrient. Anthropological research shows that the length of the gut in primates and humans became shorter with the introduction of animal-derived food. Smaller quantities of food of high digestibility required relatively smaller guts, characterized by simple stomachs and proportionally longer small intestine, emphasizing absorption (Aiello and Wheeler, 1995). It is perhaps due to the fact that meat has been eaten as much for enjoyment as for its nutritional qualities that consumption of meat and meat products has increased with the affluence of the consumer. This conclusion is consistent with the meat consumption data in selected countries. As shown in Table 2.1, it is predicted that these values will not change substantially within the next decade.

Enser *et al.,* (1996) indicated that the fat content of meat as consumed is around 2% to 5 %, even through total fat content varies with species, feeding regimes and age. The principal fatty acids in meat are saturated fatty acids, including palmitic acid (C16:0) and stearic acid (C18:0). Around 40% of the fat in meat is monounsaturated, of which oleic acid (C18:1) is one of the main contributors.

Hambreaus (1999) reported that the requirement for iron is one of the most lifficult nutritional requirements for human to be met, because iron deficiency is ;aused not only by a low intake but is also the result of low bioavailability. Increased ron requirements may result from physiological variables or clinical problems. Red neat contains 50% to 60% of its iron in the heme form (from hemoglobin and nyoglobin), which is absorbed in humans by a more efficient mechanism than ıonheme iron, the source of iron in plant foods. An important role of meat and meat ›roducts in everyday food culture and consumer health may be questioned by the fact hat the populations of vegetarians living in rich countries are characterized by lower ates of cancer and cardiovascular disease (Willett, 1999). The analysis of dietary ›atterns, as a possible approach to examining diet- disease relations, identified two najor eating patterns defined by factor analysis using dietary data collected from food requency questionnaires (Hu *et al*,. 1999). The first factor, the "prudent dietary ›attern" was characterized by a high intake of vegetables, fruits, legumes, whole ;rains, and fish or other seafood, whereas the second factor, the "Western pattern" ihowed a high intake of processed meat, red meat, butter high-fat dairy products, eggs ınd refined grains.

**Гable 2.1**: Meat consumption in 2006 and predicted values for 2015 (Kg per person ›er year)

| Country | 2006 | 2015 |
|---|---|---|
| United State | 100.63 | 103.36 |
| Canada | 77.13 | 81.74 |
| Australia | 85.64 | 85.00 |
| European Union | 69.06 | 72.11 |
| Japan | 34.12 | 36.64 |
| China | 46.24 | 55.33 |
| India | 4.26 | 4.94 |
| African contries* | 10.57 | 10.82 |

* Republic of South Africa not included.
Source: Hammes *et al.*, 2008

Meat is extremely susceptible to microbial spoilage. Virtually all ecological actors characterizing meat as a substrate are optimal for the growth of bacteria, which ıre the most efficient agents in demineralization of organic matter. For example, in meat, water activity and pH are 0.96 to 0.97 and 5.6 to 5.8, respectively, and nutrients and growth factors are abundantly available. Any storage of this nutritionally rich food and preservation of the nutrients contained therein requires the suppression of microbial growth or the elimination of microorganisms and prevention of recontamination. The traditional methods employed for prevention of microbial spoilage are still in use, though with a different meaning in the various products. These methods comprise reduction of water activity (drying, salting) and pH (fermentation, acidification), smoking, storage at refrigeration or freezing temperatures, and use of curing aids (nitrite and nitrate). Commonly, these methods act together in different combinations, building up hurdles against microbial growth. The understanding of these ecological factors and their control is not only a prerequisite in quality assurance, but also provides a basis for understanding to what extent these food matrices might be used to serve as probiotic food.

## 2.2 Composition of nutritive value of meat and meat product

The composition of meat can't be described simply in terms of the different component and their percentage, since meat includes the carcass along with the muscles, fatty tissues, bones, tendon, edible organs and glands. This gives a wide range of components and thus of composition and nutritive value. Meat is composed of water, fat, protein, minerals and the small proportion of carbohydrates. Water is the most variable of these components, but is closely and inversely related to the fat content and lesser extent to the ash and carbohydrate content. In general terms, if the fat content is held relatively content, the percentage of water declines until the animal body reaches chemically maturity. As animals mature, the also usually increase in fatness, which causes an even greater decline in the percentage of water. The inverse relationship between fat and water is the influence of fatness on composition. Animal fat is composed chiefly of natural fat and phospholipids. The natural fats are principally glycerol ester of straight chain carboxylic acids or triglycerides. The structural formulas in which all three R groups may be the same or different phospholipids normally occur in meat as phosphor-glycerides. They normally comprise about 0.5-1 % of lean muscle. As the total lipid in a muscle, decrease from 5 to 10 % the percent of phospholipids, to total lipid increases from less than 10 % to nearly 70 % since phospholipids are more readily oxidized than triglycerides, they play an important in the development of off flavors and undesirable odors in meat products. The major contribution of the fat in the diet is energy or calories. This is true because fat has 2.25 times as much energy as an equal quantity of carbohydrate or protein (Pearson and Gillett, 1999). Three fatty acids are considered essential for human's linolenic, linileic and arachidonic acid.

Three types of proteins, namely (i) Sacroplasmic (ii) Myofibrillar (iii) Connective tissue is present in meat. The nitrogenous components of meat are probably the most important. These compounds are divided into protein and non-protein nitrogen (NPN). Non-protein nitrogen exists chiefly as free amino acids and amides. Sacroplasmic proteins are often referred to as water- soluble protein, because they are commonly extracted with water or low ionic strength salt solution. The fraction contains the oxidative enzymes, including the cytochromes, the Flavin nucleotides, the various heme pigments, and the mitochonorial oxidative enzymes. The sarcoplasmic fraction also contains the glycolytic enzymes, which control both aerobic and anaerobic glycolysis, thereby functioning in the conversion of glycogen to lactic acid and the aerobic oxidation of pyruvate. In addition, the sarcoplasmic classification also contains lysosomal enzymes and nucleoprotein, which function in hydrolytic degradation of waste materials and regulates protein synthesis and deposition. Myofibrillar protein is also known as contractile proteins by virtue of the key role they play in muscle contraction and locomotion in the living animals. After the death these proteins function in the development of rigor mortis, which is essentially an irreversible reaction limited by the unavailability of substrate. The principle proteins in the myofibrillar fraction include myosin, actin, and the combination from of actomyosin, which results from contraction of muscle or in the case of meat during development of rigor morits. In addition, the myofibrillar fraction includes tropomysin, troponin, the actinins and perhaps other minor regulatory proteins, which play major roles in muscle and meat. Connective tissue proteins function as a supporting framework of the dividing body, and thus serve in numerous and varied functions. These fractions include two distinctly different proteins, collagen and elastin, and also probably another, reticulin, which is, the less well-defined than the former two. Collagen is the principle component of the connective tissue fraction. It is found widely distributed in the body and comprises 20-25 % of the total protein. It is the principal protein in bone, tendon and skin. It also comprises the protein matrix for deposition of depot fat and supports and contains individual muscle fibers, the bundles of fibers, and the muscles themselves, linked together by means of peptide linkages to form complete proteins. Meat proteins also frequently contain sulphur, and a few contain phosphorous and iron. The nitrogen content of meat protein is about 1.6 %, which means the protein content of meat is 6.25 times of the nitrogen content. Muscle or meat proteins can be divided into three different fractions on the basis of function and solubility (1) Sarcoplasmic or water-soluble (2) myofibrillar or salt soluble (3) connective tissue or insoluble.

Immediately post-mortem, muscles normally contain a small amount (about 1%) of glycogen, most of which disappears before completion of rigor. After completion of rigor mortis, the amount of glycogen is usually greatly reduced or in many cases completely absent. The amount of remaining glycogen of post-mortem meat is quite low or even completely absent, so it has little effect on the nutritive value of meat and meat products. The carbohydrate contents of meat and meat products are usually negligible unless it is added during processing either as sugar or as other carbohydrate materials. The glycogen present at the time of slaughter, although it comprises only about 1% plays a major role determining the physical properties of meat (Ahmad, 2005).

The vitamins are one of the six essential types of foods. Spatially, they are a group of organic compounds, which are required in the diet in milligram or microgram amounts. Although the vitamins are perform essential functions in humans serving as coenzymes in important life processes and in a variety of other body functions. The vitamins can be classified as fat-soluble and water soluble. The fat soluble group includes vitamin A, D, E, and K the water soluble group contains the B complex and is poor in the fat soluble group and vitamin C, liver and kidney contain appreciable percentage of vitamin A, C, D, E and K. Muscles are a poor source of A, C, D, E and K the small quantities present in fresh meat being largely destroyed during cooking and/or processing. The vitamin content of meat is quite variable being dependent on the species and age after animal, the degree of fatness, and the type of feed furnished to the animal. Most of the vitamins in meat are relatively stable during cooking or processing, although substantial amounts may be leached out in the drippings or both. Thiamine and to a lesser extent, vitamin $b_6$ is heat liable. These vitamins are partially destroyed during curing, smoking, cooking, canning, heat dehydration and irradiation. Ionizing radiation causes losses as high as 60% for thiamine. Average losses of thiamine during cooking and processing of meat and meat products amount to about 25% ionization radiation on may destroy most of the vitamin K, 25% of the riboflavin and 10% of the niacin the concentration of vitamin and in cooked meat and meat products is often higher than in raw meat (Amer, 2011).

Meat also contributes a significant percentage of number of other minerals, including iron, zinc, copper, sodium, potassium, magnesium, phosphorous, cobalt, etc. Ash content accurately reflects the mineral content but does not differentiate between minerals. Aside from bone or minerals added as of curing salt or for seasoning the mineral content of muscle is relatively constant. Because of the relatively low content of the mineral in fatty tissue, the farthest level also indirectly influences the mineral or ash content due to the concentration of the natural minerals. Since curing salts are commonly used in most dried sausage or meat products. The added salt is also concentrated and thus contributed to the total mineral content. Meat is a good source of dietary phosphorous and iron, but is low in calcium. Meat is not only a rich source of dietary iron, but that it enhances iron absorption from other sources. The iron content of meat products is particularly important in providing a readily available source of iron in the diet of no pregnant pre menopausal women, who have a recommended daily allowance (RDA) of 18 mg per day.

### 2.3 Meat Quality

Meat quality is one of the important factors which have an effect on meat utilization. Two overall types of quality can be noted. One is functional quality which refers to the desirable attributes in a product, such as tenderness, appearance (color and water-holding capacity), flavor and deliciousness. Another one is a conformance quality that refers to a product which meets the consumer specification exactly, such as portion-sized chicken breast meat to weigh exactly a certain amount. Processors interpret quality in terms of yield, uniformity, and compliance to the processing standards or product specification; whereas, consumers evaluate food quality with respect to appearance, texture, status, wholesomeness, and perceived value (Fletcher, 1999). Various tenderizing methods are available; meat tenderness is probably the most important factor influencing consumer's eating satisfaction. However, variation and inconsistency in the meat tenderness have been major problems facing both the consumers and producers. The problem of inconsistency in the meat tenderness has been partly due to the inability to regularly produce tender meat and to identify carcasses producing tough meat. Meat of spending layer fowl becomes objectionably rough with increasing age and this toughness limits its use in whole muscle foods, resulting in considerable economic losses to the poultry industry, which produces spent fowl as a by-product of table and hatching egg production (Iqabal *et al.,* 1999). For this reason, spent fowl meat has been traditionally used in less profitable, comminuted or retorted products in which small particle size or thermal processing is used to reduce the toughness (Nurmahmudi and Sams, 1997).

Studies by Locker (1960), Locker and Hagyard (1963), Marsh and Leet (1966) and Marsh *et al.,* (1968) had provided the basis for a greater understanding of the machinery by which numerous ante mortem and post mortem factors contribute to meat tenderness. For quite some time, it was believed that the connective tissue component of muscle was the primary intrinsic determinant of tenderness. However, the previously cited studies have demonstrated that the tenderness of meat, particularly that of young animals, is largely dependent upon the contractile state of muscle during the onset of rigor mortis. According to Marsh *et al.,*(1968) the contractile state and subsequent tenderness of the investigated muscle is dependent, to a large extent, upon the cooling rate of carcass tissue during the first few post mortem hours. They concluded that the cooling rate of muscle is determined not only by ambient temperature, humidity, and air velocity, but also by the size of the cooling body and the thickness of tissue covering the muscle. May *et al.,* (1962) illustrated the importance of the site of measuring broiler carcass temperature. They reported that the center of the breast required the most and drumsticks the last time to cool. Furthermore, Marsh and Leet (1966) indicated that a slightly greater thickness of fat, the proximity of a bone, or the protective shielding of a muscle from cold, and moving air may provide sufficient insulation to alter the rate of cooling and disease the extent of cold-induced myofibrils shortening and toughening. Lack of tenderness, of breast meat is a problem for convenience food and further processing operations. There is considerable interest in determining the effect of precooked treatments on the tenderness and yield of the cooked product. Wenham *et al.,* (1973) using mutton and lamb carcasses, postulated that larger, heavier carcasses produce more tender meat cuts due to the fact that they chill more slowly and, as a result, are less susceptible to cold induced toughening.

It appears that intensive pre slaughter feeding exerts an indirect influence on meat tenderness via its effect on carcass weight, fatness, and postmortem chilling rate. The rate and extent of pH drop in post mortem muscle is extremely important from the standpoint of muscle tenderness. Most authors agree that a high ultimate muscle pH (6.0 or greater) is related to a more tender muscle (Dransfield, 1981; Dutson *et al.,*1981). Several authors have also observed that certain ante mortem treatments accelerate pH drop, resulting in residual toughness in poultry muscle (de Fremery and Poole, 1963; Kahn and Nakamura, 1971). The decline in pH relates to the conversion of glycogen to lactic acid. Heat stress hastened the rate of postmortem glycolysis, but was unsuccessful in affecting tenderness (Wood and Richards, 1975). Lee *et al.,* (1970) reported that heat stress, increased the toughness of breast muscle significantly. As mentioned before, heat stressed broilers had higher concentration of glycogen and higher pH than the control birds, but those values were significantly lower than the control birds. Another considerable muscular factor is muscle types. The ratios of slow twitch oxidative and fast-twitch glycolytic muscle fibers also evidently influence meat tenderness. The ratio varies among individual animals of the same breed, breeds, and crosses. Beef tenderness is positively related to type I muscle and negatively related to the others. These differences are linked to a higher ratio of protein turnover in tender muscle and higher level of calpain (Lawrence and Fowler, 2002), which plays an important role in protein degradation, so the meat tenderness.

**2.4 Use of Antioxidant in meat and meat products**

Lipid oxidation in general and specially oxidation of phospholipids has been found to be responsible for the characteristics rancid off flavor developments in precooked meat (Ladikes and langovois, 1990). The lipid oxidation process leads to discoloration, drip losses, off odors of flavor development and the production of potentially compounds (Gray *et al.,* 1996). The oxidation effects on proteins, peptides and amino acids can impair texture, flavor and nutritional value (Spainer *et al.,* 1992). Lipid oxidation is important to the meat and poultry industry because it is one of the major causes of quality deterioration (Raghavan and Richards 2007). Lipid oxidation can impart negative effects on sensory attributes such as color, texture, odor, and flavor, as well as negatively impacting the nutritional quality of the product (Nunez de Gonzalez *et al.,* 2008). During cooking, these oxidized lipids produce secondary oxidative compounds such as hexanal, pentanal, heptanal, octanal, and secondary volatile aldehydes, which contribute to the development of Warmed Over Flavor (WOF) (Rojas and Brewer, 2007; Karre, 2009). Processed meats are highly susceptible to lipid oxidation because they are commonly ground, they are often cooked, and they generally contain salt (Rojas and Brewer 2008; Karre, 2009).

The low oxidation stability of meat and precooked and restructured meat products is a problem for all those involved in the meat production chain, including the primary producers, processors, distributors and retailers. Understanding and controlling the processes, which lead to lipid oxidation, is a major challenge for meat scientists. Sodium Pyrophosphate (SPP) Sodium Tripolyphosphate (STPP) Disodium Phosphate (DSP), Sodium Hexameta Phosphates (SHMP) or combination of these phosphates are permitted to use in long varieties of meat products such as cured meats, restructured products, sausage and poultry meat to improve their quality. Legal limits for added residual phosphates are at 0.05% of the finished products as per USDA (1982).

Polyphosphates (PP) are added in the formulation to increase the water holding capacity (WHC) and, thereby, the yield of the finished products. Hamm (1970) summarized the effect of PP on the increase in water binding capacity (WBC) in meat products as being due to (1) Increase in pH and ionic strength. (2) The ability to chelate divalent metal ions. (3) Ability to bind meat proteins. (4) The ability of phosphates to dissociate actomyosin into actin and myosin. Addition of sodium chloride (NaCl) to meat products increases pH, WHC, the binding properties of proteins to improve texture, flavor etc. (Terrel, 1983). Phosphates will be most effective when used in conjunction with 1 to 2.1% salt (Trout and Schmid, 1983). The combination of NaCl and inorganic phosphates has a synergistic effect in improving the quality of meat products. The phosphates helps to increase water retention by decreasing the amount of salt needed to generate fiber swelling (Offer *et al.,* 1983).

Pyro tripoly and hexa metaphosphate has an effective antioxidant effect (Tims and Walts, 1958). The PP provides protection against development of low as 0.01 - 0.05% (Watts, 1962). Sodium chloride acts as Pro-oxidants in processed meat products by accelerating oxidative reaction of unsaturated lipids causing increased rancidity (Gray, 1978). Use of phosphate decreased off flavor and rancidity development in many meat products and the pro-oxidants effect of NaCl was marked by the antioxidants properties of PP (Huffman, *et al.*, 1987). However Schwartz and Mandigo (1976) reported a synergistic effect of NaCl and PP in retarding oxidative rancidity and enhancing sensory traits of restructured pork. The reduction in antioxidation is probably related to the complexion of those heavy metal contaminants in the curing salts and binding of ferrous ion by the phosphates since the ferrous ion is an active oxidant in the cooked meat (Pearson and Tauber, 1984). The PP is ineffective inhibitors of lipid oxidation in raw meat, presumably owing to hydrolysis by muscle phosphates (Greene, 1969). This can occur even during freezing, storage of ground beef on -20°C (Molins *et.al.,* 1987). Trained panel rated color desirability of raw finished steaks containing salt, STPP or salt with STPP, superior to that of the controls containing no additives (Huffman *et al.,* 1981).

Studies on foods and microbial media indicating that same phosphates such as Sodium Acid Pyrophosphates (SAPP) or a blend of phosphates under certain conditions may have potential value for improving microbial or botulinal safety and stability of certain meat products (Madril and Sofo.1986). Some studies have shown that phosphate did not retard the growth of microorganism. Microbial growth and product spoilage were more rapid with decreasing bring level irrespective of the presence or absence of 0.36% Sodium Tripoly Phosphate (STPP) in comminute meat products during storage at 20°C (Sofo, 1985). However STPP significantly inhibited aerobic and anaerobic bacteria, including Clostridium sporogenes PA-3079 upon temperature abuse storage (24°C) followed by SPP and STPP further studies indicated that higher concentration of SAPP (1%) addition to fresh ground pork finished psychotropic bacteria growth ($P<0.01$), which provides 50% longer shelf life than control samples or meat treated with 0.05% SAPP or 1% orthophosphates (Molins, *et al.*, 1987).

Several studies have shown the antimicrobial and antioxidant potential of spices and herbs, such as basil, thyme, rosemary, garlic, clove, coriander, ginger, mustard and pepper (Sebranek *et al.*, 2005; Tipsrisukond *et al.*, 1998). Mint (*Mentha spicata*) is an herb used extensively in Indian cuisine and also for curing several common ailments (Choudhury *et al.*, 2006). Mint extract did not show any antibacterial activity, though essential oils of some *Mentha* species have been reported to have antibacterial activity (Marino *et al.*, 2001; Moreira *et al.*, 2005). Kanatt *et al.*, (2008) demonstrated that the efficacy of chitosan and mint mixture as a potent antibacterial and antioxidant agent that can be used for the preservation and shelf life extension of meat and meat products. Use of mint and chitosan in meat products is well suited to improving shelf life and safety of meat and other flesh foods. Dewi *et al.*, (2010) reported that fresh garlic and garlic powder provide antioxidant and antimicrobial benefits to duck sausage during refrigerated storage (4° C).

**2.5 Physico-chemical properties of meat and meat products**

Basic information on physico-chemical and functional properties of buffalo meat are necessary for its effective utilization in the development of products formulations and to evaluate its quality. The pH, water holding capacity, emulsifying capacity and cooking release volume of buffalo meat depends on holding and processing conditions. The emulsifying capacity (EC) is significantly high in head and high meat. Head meat is better than tripe and heart and has comparable merit as that of high meat for incorporation in comminuted meat products (Kondaiah *et al.*, 1986). Addition of salt (2.5%) and sodium hexametaphosphate (0.5%) to minced buffalo meat had significantly increased the pH, emulsifying capacity and decreased the cooking losses and remarkable enhanced the extractability of water and salt soluble proteins (Kondaiah *et al.*, 1985). The EC of salt soluble protein from the muscles of buffalo is higher and more efficient than of sheep and goat (Turgut, 1984). Buffalo meat is stated to have physico-chemical, biochemical and technological properties comparable to those of beef (Kandilov *et al.*, 1977). The ultimate pH of buffalo meat ranges between 5.4-5.6 (Robertson *et al.*, 1984). Glycogen content in the buffalo muscle various from 0.9–1.3% depending upon age and declined with advancing age (Yadav and Singh1985).

The rate of pH fall depends on temperature (Marsh and Thompson, 1985). When time to develop rigor morits, increasing linearly with fallen temperature (Honikel *et al.*, 1981). It is reported that the development of rigor morits in the post morten pH range (5.9-5.5) has no significant effect on the water holding capacity of dissected muscle or unsalted homogeneous prepared at different times of post-mortem irrespective of conditioning temperature. However, the development of rigor mortis in the intact muscles results in a remarkable decrease of water holding capacity (WHC) of salted muscle. The pH value of meat is reported to increase by about 0.3 units on cooking (Fogg and Horrison, 1975). Changes in pH level have been found to affect both WHC and the emulsifying capacity (EC) of meat (Helledoorm, 1962). The EC of water soluble protein (WSP) is max at pH 5.2 and sharply decreases in either in alkaline or acidic solution (Swift and Sulzbacher, 1963). It is also reported hates of salt soluble protein is max at 6.0 to 6.5 and does not change with increasing pH. At pH 8.0 the amount of protein extracted also increased with the rise of pH. (Saffle and Galbrath, 1964). Higher muscle pH values are associated with a more tender meat and the toughness of hot boned meat might be due to acceleration in pH fall (Peterson and Lily blade, 1979). Higher pH values resulted in a greater bind in fresh pork patties, higher cooking yield and improved juiciness (Keeton, 1983) and decreased in Thiobarbituric acid (TBA) values in fresh pork products (Judge and Aberic, 1980). Many researchers (Castellini *et al.*, 2006; Qiao *et al.*, 2002; Lonergan *et al.*, 2003; Wattanachant *et al.*, 2004; De Marchi *et al.*, 2005; Díaz *et al.*, 2010) reported that the lipid content of the breast and drumstick meat of capons was higher than that of broilers and organic chickens as reported. In drumstick meat, the pH values were higher than the breast meat ones; this result coincided with those previously reported in chicken meat and is probably due to the different type of muscles that predominate in the drumstick (Castellini *et al.*, 2002; De Marchi *et al.*, 2005; Díaz *et al.*, 2010).

**2.6 Buffalo and buffalo meat production**

Buffalo, a triple purpose animal, provides milk, meat and mechanical power to mankind. Due to its highly nutritious milk, leaner meat and best draught power for wet environments buffalo offers immense potential for the improvement of livelihood (Pasha & Hayat, 2012). Indian sub-continent is the home tract of world buffaloes. Buffalo is the only potential animal that can boost the meat industry in India. Buffalo meat is the healthiest meat among red meats known for human consumption since it is low in calories and cholesterol. Buffalo meat is well compared to beef in many of the physicochemical, nutritional, functional properties and palatability attributes (Anjaneyulu *et al.*, 1990). Buffalo meat has gained importance in the recent years of its domestic usage and export potential. Buffalo meat is comparable to beef in many of its physicochemical, nutritional and functional properties and sensory attributes. Buffalo meats use in meat processing is increasing, because of its higher content of lean meat and low fat. This dark meat possesses good binding properties and is useful in product manufacture. The production of buffalo meat has high growth possibilities and poses a minimal level of risk from pesticides and veterinary drugs when compared to beef production in developed countries. Buffalo meat is produced primarily in Asia. The contribution of buffalo meat to world total meat production is only 1.3 percent. India produces 1.43 million tonnes of buffalo meat annually and accounts for 36 percent of total meat production contribute significantly to human nutrition (Badpa and Ahmad, 2014a). As the meat produced is mainly from spent animals, it is coarse and fibrous. The demand for buffalo meat is high as it is relatively lean with a fat content below 2 percent and it is free from Mad Cow Disease as the animals are only fed grass and farm by-products. The functional proprieties of buffalo meat for product processing could be improved by increasing its popularity in the Indian market. For these reasons the future potential for buffalo meat and meat products is promising for India both on the domestic and international markets (Murty *et al.*, 2003). Meat from buffalo is called by various terminologies in different countries, according to the age of slaughter. Buffaloes have a unique ability to utilize coarse feeds, straws and crop residues converting them into protein rich lean meat. The carcass composition varies with a dressing percentage of buffalo carcasses. The dark buffalo meat possesses good binding properties and is preferred in product manufacture. Buffalo meat is the major item of Indian animal product export. Adequate nutrition and improved levels of hygiene at meat handling will enable India a quantum jump in meat production by utilizing the surplus male calves. Massive developmental programs have to be launched to produce meat as the principal commodity from buffaloes for substantially increasing the livestock economy of the country.

### 2.6.1 Buffalo inherent qualities as to produce meat

Buffaloes have a unique ability to utilize coarse feeds, straws and crop residues converting them into protein rich lean meat. Hence buffaloes fit well in poor countries having poor feed resources (Arganosa, 1973). Buffalo properly managed and fed as a meat producing animal and slaughtered at 16 to 20 months of age yields a highly satisfactory top quality meat at a much lower cost than the cattle (Ranjan and Pathak, 1979). Buffaloes are lean animals. The sub-cutaneous fat layer of the carcass is usually thinner than that of comparably fed cattle. Fat is low even under feed lot conditions (Desmond, 1990). More lean and less fat compared to cattle, has created a demand for it among health conscious consumers (Kondaiah, 2002). Buffaloes have a higher degree of resistance and tolerance than cattle against many diseases (Ross, 1975).

### 2.6.2 Physicochemical and functional characteristic of male and female buffalo meat

Buffalo meat is dark red in color, firm in consistency with white fat color (Joksimovic and Oqnjanovic, 1977). More pigmentation or less intramuscular fat (1-2% marbling compared with 3-4% in beef) content causes darker appearance of buffalo meat. The dark meat possesses good binding properties and is preferred in product manufacture (Kondaiah, 2002). β-carotene in the fat gives yellow coloration and is totally absent in buffalo fat (Joksimovic and Oqnjanovic, 1977). Buffalo meat constitutes higher protein, low fat and cholesterol (Arganosa, 1973; Ross, 1975; Joksimovic and Oqnjanovic, 1977; Anjaneyulu *et al.*, 1994; Kandeepan and Biswas, 2007a). Buffalo product presents healthy physicochemical characteristic respect to Beef product, especially in terms of protein, fat and iron contributions against securities FAO (Eyas *et al.* 2007) and ICBF (FAO) requirements. The normal ultimate pH of buffalo meat varies from 5.4 to 5.8 (Kaneppan *et al.*, 2009). The pH of the meat from intensively reared young males was 5.57, which did not differ significantly from spent male buffalo meat. The significantly ($p<0.05$) lower ultimate pH is spent female buffalo meat might due to the response of a female buffalo to transport stress (Jedlicka *et al.*, 1980). Table 2.2 shows physiochemical properties of meat from a different group of buffaloes.

### 2.6.2.2 Proximate composition of buffalo meat

Buffalo meat has certain attributes such as lower intramuscular fat, cholesterol and calories, high unit of essentials amino acid, biological value and iron content (Anjaneyulu *et al.,* 1990). Buffalo meat has moisture (74.2%), protein (20.4%), fat (1.4%), ash (1.0%) and water soluble proteins (5.1%), salt soluble protein (7.2%), non protein nitrogen (0.37%) and hydroxyproline (0.12%) of LD muscle from male buffalo (Lazar, 2001). The meat of intensively reared young buffalo showed significantly higher moisture content than meat from spending and female buffalo (Kandeepan *et al.,* 2009). The moisture content of buffalo meat decreases as the age of the age of the increases which is probably associated with an increase in fat content (Lawrei, 1998). Meat from spending female buffaloes had a significantly higher fat content compared to the other groups. Fat is last tissue to mature and other animals, tending to be fatter (Warriss, 2000). The meat pigment content from younger buffalo was significantly lower than that of spending male and female buffaloes. A slight variation in myoglobin concentration was observed in the meat from spending male and females; the meat becomes darker in color with increasing age (Valin *et al.,* 1994), as the meat pigment concentration increase with greater content of myoglobin (Mamino *et al.,* 1996). Spent buffalo meats have significantly higher salt soluble protein compared to the meat from young male and spent female buffaloes. Salt soluble protein was related to the water holding capacity and moisture content of the meat in each group. Meat with higher salt soluble protein can retain more water to improving the cones and binding strength of the product during processing (Swan *et al.,* 2006).

**Table 2.2**: Physicochemical and functional properties of meat from different groups of buffaloes

| **Parameters** | **Group** | | |
|---|---|---|---|
| | **Young male** | **Spent male** | **Spent female** |
| **Physicochemical characteristic** | | | |
| pH | $5.57 \pm 0.02^{ab}$ | $5.59 \pm 0.02^{a}$ | $5.52 \pm 0.01^{b}$ |
| Moisture (%) | $74.99 \pm 0.038^{a}$ | $73.42 \pm 0.28^{b}$ | $72.63 \pm 0.46^{b}$ |
| Protein (%) | $21.2 \pm 0.26^{a}$ | $21.42 \pm 0.73^{a}$, | $20 \pm 70 \pm 0.32^{a}$ |
| Fat (%) | $2.67 \pm 0.24^{b}$ | $2.76 \pm 0.25^{b}$ | $3.98 \pm 0.35^{a}$ |

| | | | |
|---|---|---|---|
| Total meat pigment | 1107.92±3.15[b] | 1148±6.43[a] | 1146.82±3.58[a] |
| Salt soluble protein (%) | 5.89±0.06[ab] | 6.04±0.09 | 5.79±0.09[b] |
| Collagen content (%) | 0.82±0.02[a] | 1.54±0.02[a] | 1.58±0.24[a] |
| Collagen solubility (%) | 29.90±1.64[a] | 7.4±0.28[b] | 9.33±0.77[b] |
| Muscle fibber diameter (μm) # | 69.83±0.73[c] | 73.47±0.9[b] | 78.87±0.90[a] |
| Sarcomere length (μm)## | 1.83±0.02[a] | 1.51±0.01[c] | 1.56±0.01[b] |
| Shear force value (N) | 60.60±2.11[c] | 81.90±1.96[b] | 93.81±1.35[a] |
| Myofibriller **fragmentation index (%)** | 84.77±0.67[a] | 79.69±0.3[b] | 72.23±1.94[c] |
| Functional properties W.H.C (ml/100g) | 13.34±0.99[a] | 12.67±1.67[a] | 8.67±0.54[b] |
| Emulsifying capacity (ml oil/2.5g meat) | 103.60±1.05[b] | 116.0 ±3. 03[a] | 106.0±3.51[b] |

n=10, #n= 375, ## n=500

Mean with different superscripts in the same row indicate significant differences ($p<0.05$).

Source: Kandeepan *et al.,* 2009

The collagen content of meat from spending female buffalo was marked higher compared to spent male buffalo meat (Kandeepan *et al.,* 2009). An age related increase in pyridinoline content of intramuscular collagen and cross link formation influenced by sex contributed to the toughness of meat in spending groups (Bosselmann *et al.,* 1995). Water holding capacity (WHC) of young buffalo meat did not differ significantly from the spent male buffalo meat. A slightly lower WHC in castrates compared to entire males has been shown to be due to higher protein denaturation in castrates (Dessouki *et al.,* 1981). Meat from intensively reared young male buffalo has higher WHC, then the meat from spending female buffaloes (Kandeepan *et al.,* 2009).

**2.6.3 Nutritional characteristics of buffalo meat**

The nutritional characteristics of buffalo meat (Infascelli *et al.,* 2003) confirmed the very low lipid content (1.36±0.1%), correlated to the lower energy value of the diet (0.84 UFC/kg DM) fed to the animals. The cholesterol content (48.8±2.9 mg/100 g) was lower than that reported for Italian bovine genotypes with an aptitude for meat production. The content of myristic, palmitic and stearic acids, the first two with both atherogenic and thrombogenic activity, was also very low. Thus, despite the low values of oleic acid and polyunsaturated acid of the ω-6 and ω-3 series, both the atherogenic and thrombogenic indexes were very low (0.53 and 1.48, respectively). Based on these results, the nutritional quality of buffalo meat can be considered of great interest.

Alkaline phosphatase (ALP) is an enzyme made in the liver, bone, and the placenta and is normally present in high concentrations in growing bone and in bile. Alkaline phosphatase is released into the blood during injury and during such normal activities as bone growth and pregnancy. An increase of ALP during early lactation in buffaloes is proof of speedy parathyroid activation (Campanile *et al.*, 1997). Pizzuti and Salvatori (1993) found differences in the ALP mean values of buffaloes at various distances from partum, increasing in the advanced phases of pregnancy and decreasing before parturition; they ranged from 159 to 228 U/l. Terzano *et al.*, (2000) reported ALP values ranging from 200 to 650 U/l, in adult buffaloes under different housing conditions. Most calcium in the body, about 90 percent, is in the bones, where it can be reabsorbed by blood and tissue, but about one percent is used for nerve impulses and muscle contractions (including the heart, kidney, and other organs). Terzano *et al.*, (2000) found 8.87-10.63 mg/dl of a blood level in adult buffaloes. In buffalo species calcium excesses could alter the Ca/P ratio during the dry milk period, inducing parathyroid hypoactivity which would cause magnesium to increase and calcium to decrease at the beginning of the lactation due to a non immediate calcium mobilization by the bones. Phosphorus is the second most plentiful "essential mineral" in the body and is a key component of DNA, RNA, bones, teeth, and many other compounds required for life. It plays an important role in the energy metabolism of cells, affecting carbohydrates, lipids and proteins. Phosphorus also stimulates muscle contraction and contributes to tissue growth and repair, nerve-impulse transmission, central nervous system health, and proper heart and kidney function. Phosphorus levels in buffaloes have been found to be quite stable at six mg/dl (Campanile *et al.*, 1997). In water buffalo mature females, inorganic phosphate was significantly higher compared to that of immature females (Canfield *et al.*, 1984). Potassium is the third most abundant mineral in the body, after calcium and phosphorus. Low bloods levels have been observed in cattle fed with high concentrate levels and have also been associated with stress conditions. In buffaloes physiologic values range are from 4 to 5 mmol/l (Bertoni, 1999).

Magnesium is involved in more than 300 enzymatic reactions. Magnesium is essential for the conversion of vitamin D to its biologically active form which helps the body absorb and use calcium. The high magnesium concentration is found in the tissues that are most metabolically active including the brain, heart, liver and kidney. Physiologic values in buffaloes were 1.1-1.3 m mol/l in the dry milk period and 1.2-1.4 m mol/l during lactation (Bertoni, 1999). Iron mainly works in the red blood cell hemoglobin, which transport oxygen from the lungs to the body's tissues, including the muscles and the brain. Irons is also a component of myoglobin, a similar protein in the muscle that stores and provides oxygen during muscle exertion and are found in the part of the cell involved in energy production and as a co-factor for several enzymes. Cavallina *et al.,* (2003) found mean iron blood levels in buffalo calves at one and three months of age of 96 and 143 mmol/l, respectively (unpublished data). In lactating buffaloes Fagiolo *et al.,* (2004) found seasonal differences in iron blood levels: higher in winter than in summer (150.07-61.81 mmol/l) though not significantly. Zinc is a part of every cell in the body and forms part of over 200 enzymes that have functions ranging from proper action of body hormones to cell growth. In buffaloes, it normally ranges from 10 to 12 μmol/l in the dry period and from 9 to 12 μmol/l during lactation (Bertoni, 1999).

### 2.6.4 Nutritional changes in buffalo meat during cooking

The nutritive value of protein from muscle is known to be very high. The muscle protein also suffers little damage when processed alone and denaturation does not affect protein quality (Bender, 1978). The proteins of the connective tissues are generally considered to have little or no nutritive value. Furthermore collagen fibers are more resistant than most other protein fibers to be attacked by proteolytic enzymes (FDA, 1985) and meat collagen is considered to be only partly digested. The low digestibility of collagen as well as of many unprocessed proteins is considered to be due to naturally occurring cross links (Cheftal, 1979). With this background the Swedish National Food Administration had proposed legislation to limit the collagen content in meat products. Nath *et al.,* (1995) reported that chicken patties were prepared by the addition of different levels of chicken fat as well as other ingredient and subjected to the boiling water and microwave oven cooking. The product yield as given in high, pH, fat and protein content differs significantly. Acceptability was better in hot air oven than a microwave oven cooked patties, the former having a significantly higher overall acceptability score. Cooking at the commercial scale or at home affects the nutrient value of meat foods, including poultry meat, nutrient changes can occur through heat liability, leaching into the cooking liquid, drip losses or absorption of cooking media (Posati, 1979). Protein and amino acids may be lost in small amounts in the drip of the cooked meat (Erdman *et al.*, 1982) but losses are not of serious concern (Mauron, 1982). Feet remain fairly stable during normal cooking of poultry (Change and Watts, 1952). An apparent increase in lipid content with cooking may be due to moisture loss, transfer of lipids from skin to the flesh or uptake of frying oil (Posati, 1979). Because poultry meat is always cooked by varieties of ways; data comparing the cooking methods may be useful in minimizing nutrient losses.

### 2.6.5 Quality improvement of buffalo meat

Most of the buffaloes are slaughtered at the end of their productive or working life and therefore, the meat is dark, coarse and tough. Now-a-days, consumers are demanding quality meat with more emphasis on tenderness which is often not obtained from the aged and spent animals. As a consequence, the majority of the meat is losing its popularity and demand. Some quantity of meat produced is used for development of various comminuted meat products. Practical methods for improving the tenderness of such meat to an acceptable level would definitely increase retail value and marketing opportunities.

Freezing of adult male buffalo meat improved texture, tenderness and juiciness scores during prolonged storage (Kandeepan and Biswas, 2007b). Electrical stimulation of buffalo carcasses significantly improved sensory tenderness scores. The tenderness improvement of more than 32% was observed in electrically stimulated carcasses (Biswas *et al.*, 2007). High voltage electrical stimulation (700 V, 1400 V peak, pulses 1 *s* on/1 *s* off, 60 *Hz*, 2 *A*) on buffalo carcasses resulted in a significantly higher tenderness scores and myofibril fragmentation compared to non-stimulated controls, irrespective of the cooling process adopted (Soares and Areas, 1995). Ground buffalo meat from spending females treated with 500 ppm sodium ascorbate significantly increased the pH, color, odor and chroma, but decreased cooking loss, metmyoglobin and TBARS number (Sahoo and Anjaneyulu, 1997a). Use of 10 ppm tocopherol acetate for preblending extended the shelf life of ground buffalo meat from 6 to 8 days under refrigerated storage (Sahoo and Anjaneyulu, 1997b). Use of 1.0% carnosine for preblending extended the shelf life of ground buffalo meat up to 8 days under refrigerated storage (Das *et al.,* 2006). Meat chunks from spending buffaloes marinated with 2% (w/w) powdered cucumis extract and 5% (w/v) ginger extract for 48 h at 4°C significantly ($P<0.01$) improved the flavor, juiciness, tenderness and overall acceptability scores (Naveena *et al.,* 2004).

## 2.7 Meat product

It must be recognized at the beginning that in the production of meat and meat products throughout the world, there are many differences of opinion among farmers, wholesaler, consumer and the meat manufacturing industry. The annual consumption of meat of any country depends upon its economic status, affluent countries consuming more than the other. The consumption of meal in India is of the order of 1.5 kg per annum. The world demand for meal is growing up steadily because of the preference based on palatability.

Indian buffalo meat production is growing significantly. (Calendar year) CY 2013 Indian buffalo meat production is thus forecast to rise to a record 4.16 million tons (on a carcass weight equivalent basis), up 14 percent from CY 2012. CY 2012 buffalo meat production is estimated at 3.64 million tons (up 12% from CY 2011), and CY 2011 production has been slightly revised up to 3.24 million tons (Singh, 2012). India has 59% of word buffalo population and it is major producer of buffalo meat. India's production of buffalo meat is 50% of the world buffalo meat production.

### 2.7.1 Traditional type of meat products

#### 2.7.1.1 Patty

A patty is a flattened, usually disc-shaped, serving of ground meat or meat alternative. The meat is compacted and shaped, cooked if applicable, and served. Patties can be eaten with a knife and a fork, in dishes like Salisbury steak, but typically serve in a sandwich called a hamburger, if made from ground beef. The patty itself is also called a burger, whether or not it's served in a sandwich (Histor, 2008).

#### 2.7.1.2 Kebab

Kebab is a wide variety of skewered meals originating in the Middle East and later on adopting in the Balkans, the Caucasus, other parts of Europe, as well as Central and South Asia that are now found worldwide. In English, kebab with no qualification generally refers more specifically to shish kebab (Turkish; "sis Kebap") cooked on a skewer. In the Middle East, however, kebab refers to meat that is cooked over or next to the flames; large or small cuts of meat, or even ground meat; it may be served on plates, in sandwiches, or in bowls. The traditional meat for kebab is lamb, but depending on local tastes and religious prohibitions, it may now be beef, goat, chicken or fish. Like other ethnic foods brought by travelers, the kebab has become a part of everyday cuisine in many countries (Histor, 2008).

#### 2.7.1.3 Shami Kabab

Shami kebab or shami tikka is a popular Iranian and Indian variety of kebab, also found in Pakistani cuisine. Shami Kabab is a delicious lamb/meat preparation, very popular among all types of Kababs in Indian subcontinent. Easy to prepare and can be stored in refrigerators for a real long time. A good accompaniment for any kind of meals and even served as snacks with evening tea. Children love them and hence Shami Kabab is a great idea for children's lunch-box too. There are different ways to make Shami Kababs but this method is the easiest one as it doesn't need minced/ground meat (Histor, 2008).

### 2.8 Sausage

Sausages are one of the oldest forms of processed foods, their origin has been lost in antiquity. It has been reported that sausages were used by the Babylonians and the Chinese 1500 BC., although documented proof for this is lacking (Pearson and Gillett, 1997). They are different sausage products available to consume. They have special appeal to some part of the population. Most of the sausages originated in Europe and were first made in the United States to meet the demand of a particular ethnic group; other population groups living in the same neighborhood frequently developed a taste for these characteristic products (Pearson and Gillett, 1997). The traditionally sausage formulation was designed by experts who based on their experience, we're able obtaining desired properties for the sausages. They were able to plan simultaneously mixture for the factory in which the carcasses were used totally without waste (Petri, *et al.*, 2004).

### 2.8.1 Classification of sausage

The term sausage covers such a diversity of products that no single classification system is completely satisfactory (Pearson and Gillett, 1997). Some of the more common classification systems are as follows:

1. Degree of chopping
   i. Coarsely ground
   ii. Emulsion of finely chopped
2. Amount of cooking
   i. Uncooked
   ii. Cooked
3. Amount of smoking
   i. Unsmoked
   ii. Smoked: natural smoke; smoke flavorings, isolated smoke components
4. Amount of water added
   i. No added water
   ii. Water added
5. Amount of curing
   i. Uncured (fresh)
   ii. Cured
6. Amount of fermentation
   i. Unfermented
   ii. Fermented
7. Amount of moisture in final product
   i. Fresh: unsmoked or smoked
   ii. Smoked: fresh and cured
   iii. Cooked: fresh and cured-smoked and unsmoked
   iv. Cured: smoked and unsmoked
   v. Meat loaves and specialty items
   vi. Dried: semi-dry and dry

Fresh sausage is prepared fresh, uncured meat, generally cuts of fresh pork and sometimes beef, their taste, texture, color and tenderness are directly related to the ratio of fat to lean. Trimming from primal cuts such as pork loins, hams, and shoulders are often used. They must be kept under refrigeration and cooked before serving, for example Bratwurst. Some products of fresh sausage are fresh pork sausage, Italian sausage and some types of bratwurst and bockwurst.

Fresh pork sausage made from fresh or frozen pork or both, including mechanically deboned pork, but not including pork bio-products. The finished products cannot contain over 50% of total fat. Water and ice can add up to 3% of the total to facilitate chopping and mixing. Fresh beef sausage same s fresh pork sausages expect beef is substituted for pork and the finished product cannot contain over 30% fat. Breakfast sausage made from fresh or frozen pork meat bio products. Extender and binder used up to 3.5% of the finished products and not contain over 50% of fat. Whole hog sausage made from fresh or frozen meat from pigs in such proportion as being present in a single animal; otherwise the same requirements applies as in the case for fresh pork sausage.

Uncooked smoked sausage have characteristics of fresh sausage just they are smoked to give the products a different flavor and color and they must be cooked before eating. Cooked sausages are comminuted, semisolid sausages prepared from one or more kinds of raw skeletal meat and poultry meat. They shall not contain more than 35% fat and 10% added water. They may be smoked or unsmoked. Some cooked sausages are Frankfurters, Bologna and Knockwurst. Cheesefurters sausages are products of resembling Frankfurters but containing sufficient cheese to give the characteristic flavor to finished products. May contain cereal, vegetable starch, soy flour, soy protein concentrate, isolate, nonfat dry milk and calcium reduced skim milk. They should not more than 3.5% in total exclusive of the cheese. Liver Sausage and braunschwelgerare made fresh or frozen pork, beef meat, pork liver, beef liver and veal livers. Liver sausage may also contain fat, beef and pork bio-products. The liver must consist of not less than 30% of the fresh weight. The binder and extender used in accordance with regulations. The product is cooked and ready to serve.

Dry and semi-dry sausages are produced by fermentation due to either natural or addition of lactic acid producing culture. The production of lactic acid not only aids in preservation by lowering the pH and inhibiting the growth of undesirable microorganisms, but added a tangy flavor. After mixing the meat ingredients with the spices, cure and culture the meat is held in a curing cooler until the desired acidity is achieved. Then the meat is stuffed into casings and air dried under controlled drying conditions. Some products are smoked during preliminary drying, but the essential step is air drying.

**2.9 Bacterial safety and hygiene of sausage**

Microbial hazards are a major concern in the production of food of animal origin. Meat and meat products can be contaminated with bacteria during manufacturing and packaging. Microorganisms gaining access into sausage from meat, spices, and other ingredients, from environment, equipment, and handlers during processing affect the microbiological status of the products.

Ebru *et al.,* (2010) found in minced meat sausage batter, and stuffed sausage were no significant ($P<0.01$) changes in total plate count and S. aureus counts between minced meat and sausage batter but significant ($P<0.01$) increases were observed in E. coli and yeast-mold counts. Total plate count in stuffed sausage was higher ($p<0.01$) than those of minced meat and batter. There were no significant ($p>0.01$) differences in S. aureus counts of stuffed sausage compared to minced meat and batter. E. coli and yeast-mold counts of stuffed sausages were significantly ($p<0.01$) different than those of minced meat but not significantly ($P>0.01$) different than those of batter.

Microorganism counts of cooked sausage, which was cooked after stuffing at 76 °C for 25 min, Total plate and S. aureus counts of cooked sausages were significantly ($p<0.01$) lower than those of minced meat, batter, and stuffed sausage. E. coli and yeast-molds were not detected in cooked sausages. There were no differences in microbial counts of sausages after the peeling process. Total plate and S. aureus counts did not change after peeling. E.coli and yeast-molds were not detected in peeled sausages. After the peeling process the sausages were packed in polyethylene bags under vacuum and pasteurized at 85°C for 10 min. Total plate and S. aureus counts in pasteurized sausages were significantly ($p<0.01$) lower than those of cooked and peeled sausages (Ebru *et al.,* 2010). The TPC value of cooked sausages prepared with different levels of added chicken fat skin were statistically non-significant ($p<0.05$) and that the shelf life of ground chicken sausage influenced by bacterial spoilage was unaffected by fat content (Young *et al.*, 1991).

### 2.10 Emulsion

In the culinary arts, an emulsion is a mixture of two liquids that would ordinarily not mix together, like oil and vinegar. In a meat emulsion, fine fat droplets are dispersed in an aqueous medium containing soluble proteins, other soluble muscle constituents, segments of muscle fibers and connective tissue fibers. In a stable emulsion, each fat droplet is coated with a thin layer of soluble protein which has been released into the aqueous medium from the muscle fibers. Soluble proteins act as emulsifiers to stabilize fat and moisture. When fat is not stabilized, fat separation occurs. Meat emulsions arc finally comminuted meat mixtures composed of water, protein, fat, salt and small amount of other ingredients. Emulsion sausage like frankfurters and bologna are important part of diet in developed nations (Alvarez *et al.*, 2007). Chopping is one of the most steps in meat emulsion manufacturing.

During this process, the raw materials are extensively comminuted in a bowl chopper in order to reduce the size of the particles and obtain a stable and homogeneous emulsion. At the latest stage of the cooking process, when the temperature reaches 70–75°C, fat tends to melt and expand (Townsend *et al.*, 1968), collagen is transformed into a liquid-like gelatin and myofibrillar proteins coagulate (Samejima *et al.*, 1976), causing a significant reduction in the water-holding capacity of proteins (Shults *et al.*, 1972). Salt soluble protein denaturation after cooking turns the emulsion into a viscoelastic gel matrix (Xiong, 1997).

Meat proteins serve as the natural emulsifying agent in a meat emulsion. To form a stable meat emulsion, these proteins must surround the finely chopped fat particles before cooking. Myosin is the most important of the proteins for fat emulsification and water holding capacity of processed meats that may bridge the oil–water interface during the emulsification steps (Xiong, 2000) thus, as fat particle size decreases during chopping the emulsion stability will increase, provided there is sufficient protein to coat all the fat particles. As chopping continues, emulsion temperature increase causes the surface tension of the fat particles to decrease. This

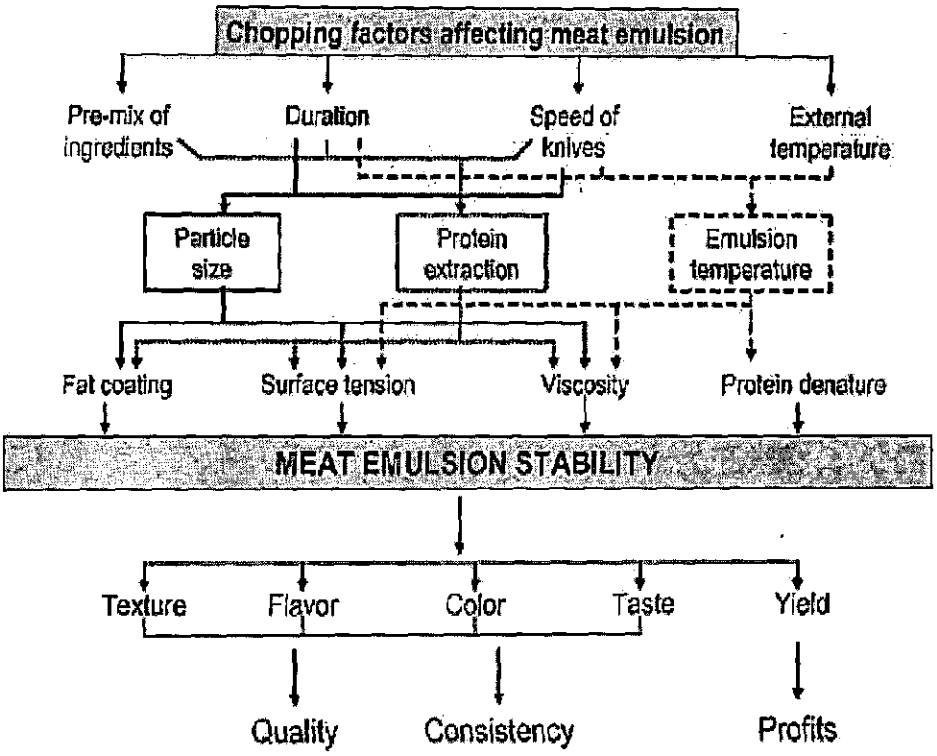



**Fig. 2.1:** Chopping factors affecting meat emulsion stability (Source: Alvarez *et al.*, 2007)

### .10.3 Manufacturing of emulsion sausages

#### .10.3.1 Choice and preparation of raw meat

The meat must be fresh or frozen. The lean meat should be well trimmed to a ɜvel of less than 10 percent of non trimmable fat and connective tissue, the trimmed ɜan meat thus being practically free from sinews and gristle and entirely free from igament, bone and cartilage particles. The selection of meat should be such that most ıeat ingredients are of a good water binding capacity. About 20% of fat is needed for ;ood texture, taste and flavor. Hard and soft fats can be used. Pork fat, beef fat, nutton fat, chicken fat or even vegetable oils can be utilized. Beef and lamb fat have a 'ery strong flavor which can be masked by a careful choice of spices. Buffalo meat is ɪne of the best sources for developing of sausages. Buffalo meat has been used for ɪrocessing of products like sausages (Sachindra *et al.*, 2005), loaves (Suresh *et al.*, !004) burgers (Modi *et al.*, 2003), patties (Suman and Sharma, 2003) and nuggets Thomas *et al.*, 2006). Inclusion of non meat additives as binders or extenders had ɪeen documented in production of emulsion based meat products (Dushyanthan, 2008). Meat from the lean cuts of female buffalo were cut into smaller cubes of 2.5cm thickness, frozen at -18°±1°C and utilized in the product manufacture. The emulsion was prepared utilizing 80% of meat and 20% of fat (10% replaced with refined sunflower oil) (Dushyanthan, 2008).

### 2. 10.3.2 Comminution

The raw material may be comminuted in cutters or grinders; the latter brings about shorter (more crumbly) consistency required for various traditional products. It is important to keep the temperature close to the freezing point; otherwise the efficiency of cutting will be reduced and fats may smear. It is common practice to add part of the raw material in the frozen state.

### 2. 10.3.3 Additives

The addition of 2.5 to 3% (W/W) salt (sodium chloride) is most common. In conjunction with a moisture content of the mix of about 50% (as in common formulations), this will give an initial $a_w$ of 0.0955- 0.965. Apart from its effect on the micro flora and flavor, salt interacts with the myofibrillar structure and solubilizes proteins, which from a sticky film around meat particles. Nitrite is preferred as curing agents, for undried and semi-dry sausage with maximum input levels about 150 mg $NANO_2$ $kg^{-1}$. In many countries, nitrite may only be applied as a mixture containing about 0.5% sodium nitrite in NACL. This effectively prevents overdosing of nitrite because such products would taste too salty. Sodium ascorbate (usually 300-500 mg $kg^{-1}$) is frequently added, together with nitrite to accelerate the development of curing color and flavor. Also use nitrate up to (300 mg $KNO_3$ $kg^{-1}$) for reducing levels of nitrite. Ground paper is present of all types of sausages at a concentration of 0.2% to 0.3%. Other spices commonly used include paprika, garlic, mace, pimento and cardamom. Generally, air dried sausages are more heavily spiced than smoked sausages. Apart from their effects on flavor, spices may also affect the micro flora and the oxidative changes during ripening.

### 2. 10.3.4 Grinding

The grinding of meat and fat ingredients has largely been practiced for many years and is still done today, mainly by small processors, particularly in the manufacture of specialty products. The fist-size chunks of lean meats are first ground by running them through a 3–6 mm grinder plate while fat trimmings or fatty tissues are reduced through a 6–9 mm grinder plate. The tripe and the filler meats are preferably ground twice: first, through a 3–4 mm and then through a finer grinder plate. Grinding through a coarser plate increases the capacity of the machine and heats the meatless. Particularly in bull meat, grinding through a finer plate is considered to give a product with better binding and emulsifying properties. The curing salts are then added and the batch is mixed in a mechanical mixer to ensure that the ingredients are well dispersed. The curing process may take place either overnight in a chiller at 1–4°C (this practice is being increasingly abandoned) or after final chopping in the cutter with other ingredients and stuffing, i.e. prior to or during the smoking. During many emulsion-type sausage procedures, a pre comminution in the grinder is followed by chopping because it contributes to a better and more uniform size reduction in the cutter. In many cases comminution is not too finely done.

**2. 10.3.5 Chopping**

The grinding of meat has by large been replaced by cutter chopping which renders a fine meat-fat mixture, usually known as an "emulsion". During the chopping process the meat is cut to a very fine particle size which encourages protein extraction. Proteins have the function of binding the water surrounding fat droplets and keeping them dispersed. Preparation of sausage emulsion is basically in two phases. First, the lean meat, either previously ground or not, is placed on the cutter and chopped. This is done by the simultaneous addition of all the curing salts (kitchen salt, nitrite), phosphate and/or citrate for the total batch and one-third of the total amount of finely crushed ice or water. Increased salt concentration in the water phase of the mixture will result in a greater extraction of the meat protein and is of paramount importance in forming a stable emulsion. The extraction of protein is more effective when the meat is near freezing point, but the emulsification process is adversely affected by low temperature. Since protein extraction is increasing with the time of chopping, the lean meat should be chopped for a sufficient period, normally not less than 6–8 minutes. After this time, fat trimmings and other fat meats, then spices and the remaining two-thirds of the total water are added. Chopping is then continued until the batch is thoroughly chopped or the temperature of the meat mass reaches not more than 18°C (second phase). In the course of this time, all water is taken up by the disintegrated and homogenized meats. If sodium ascorbate is used, it is also added to the very end of the chopping operation. Preparation procedures which provide for one-phase simultaneous meat and fat cutter treatment are increasingly popular. The polyphosphate and curing ingredients should be dissolved in a small quantity of warm water before being added early enough to enable the effect of polyphosphate on actomyosin and the action of salt and nitrite on water binding properties and color of the meat. With the high salt content and the longest cutter process, more salt soluble proteins are extracted and the binding quality of the finished product is improved. If hot boned meat is used, the addition of phosphates is not necessary.

**2. 10.3.6 Filling**

Before filling into casings, oxygen should be excluded from the mixture (by vacuum-filling devices) and the temperature of the mix should not exceed 2°C. Natural casing (made from the intestines of slaughter animals) as well as casings made from modified collagen or cellulose is most frequently used. Four undried products, synthetic casings are also used. Evacuation of air from the product enhances color stability and the visual effect of the sausages. It also reduces fat oxidation and bacterial action and prevents proteolysis. A longer shelf life of sausages is therefore achieved by vacuum filling (Essien, 2003).

**2. 10.3.7 Casings**

Casings, also known as skins, used in sausage manufacturing to achieve their primary significance in portioning. They are broadly divided into two types, namely natural and artificial. Intestines of pigs or sheep are used in making natural casings. Most products made of natural casings come out with a curve after filling and cooking. Artificial casings are now made with collagen, cellulose and plastic materials to suit a wide range of applications. Through a series of mechanical and chemical actions, collagen is extracted from the connective tissue of animals and used for manufacturing casings. Apart from providing the required sausage shape, casings also increase product shelf life by providing high moisture and oxygen resistant properties with a seal strength and density. Casings therefore contribute in minimizing product weight loss during cooking (Essien, 2003).

### 2. 10.3.8 Cooking

There are many methods of cooking: by immersing in the cooking vat, hot showering that is conducted in a smokehouse equipped with shower nozzles, hot showering in separate hot water spray cabinets to which sausages are moved immediately after smoking, cooking by dry heat by raising the smokehouse temperature and giving only a final brief hot water shower, cooking in tight boxes into which live steam is injected, etc. Cooking schedules vary markedly. If water sprays are used the temperature is about 80–82°C. The temperature of water in cooking vats may be about 73–76°C. A final internal sausage temperature of 65°C is considered as minimum but a temperature of 68°C is an optimum end-point temperature providing a sufficient shelf life of the product and desired organoleptic characteristics. This usually requires about 15–20 minutes. The right cooking schedule should be developed by carefully studying the yield and quality of the sausage. After cooking in vats, sausages are hot showered to remove any adhering grease.

### 2. 10.3.9 Cooling

Chilling by a cold water spray (16°C) is applied at the end of the thermal processing schedule to cool frankfurters to slightly above room temperature before placing them in a 1–4°C cooler for final chilling.

### 2. 10.3.10 Packaging

Food packaging is an integral part of food processing and vital link between the processor and the eventual consumer for the safe delivery of the product through the various stages of processing, storage, transport, distribution and marketing. The main purpose of packaging is to prevent meat and meat products from microbial contamination and physical, chemical damaged. Packaging materials for sausages whether primary or secondary should be good enough to offer an acceptable visual and structural presentation of the product to the customer. The most important criterion is probably that packaging materials are able to form a barrier against physical abuse, contamination and damage to the product. Care should be given to selection of primary and secondary packaging materials to ensure that they are food grade and that while directly in contact with the food they do not carry or transfer contaminants. Vacuum packaging is used on saveloys, frankfurters and cooked sliced sausages to prolong shelf life, and there is an increasing popularity of its use in cooked, chilled and frozen products. Vacuum packaging of sliced sausages is ideal for the sandwich and meal sectors if the packs are passed through a sterilizing or sanitizing unit before use within the process (Essien, 2003). Packaging meat and meat products with appropriate plastic film and laminates plays significant role retention of the quality and extension of shelf life during refrigerated storage. Vacuum packed preblended buffalo meat and meat products to better refrigerated storage stability (Sahoo *et al.,* 1997). Vacuum packaging buffalo meat sausages have better flavors score during refrigerated storage (Deenathyalan 1997).

### 2.10.4 Quality of emulsion sausages

Quality of sausage determined their physicochemical, microbiological and sensory characteristics. Quality of emulsion sausages very much depends upon the quality of meat used for its preparation. Evaluation of meat quality is based on two main considerations: first, meeting the requirement of meat trade and second, satisfying consumer preference. For simplicity, the characteristic of meat quality may be categorized as follows: firs, the factors contributing to the appearance of the meat must be taken into account, which include the color of lean, fat and the texture. These traits may be important in the case of fresh meat, but may be of less significance in cooked meat. Second the factors contributing to the eating quality of meat must be considered, such as tenderness, juiciness, taste and aroma (Ahmad, 2005). Microbiological quality in terms of microbial count/g $10^2$ excellent qualities, $10^4$ good commercial qualities, $10^6$ rejection limit in many commercial conditions, $10^8$ meat and meat products smell and $10^9$ meats become slimy (Ranken and Kill, 1993).

### 2. 10.4.1 Sensory characteristic of emulsion sausages

Most of the meat products produced today is based on traditional practices. These products are attractive to consumers because they offer a wide variety of colors, flavors and textures (Amer, 2011). They are many factors affecting the sensory characteristics of meat products such as the meats used as raw materials (genetic type, feed, age, sex and rearing system) microorganisms selected as microbial starters for the fermentation and type of processing technologies (cooking, drying, ripening, smoking, etc.). There are many different cured meat products although they could be grouped into two major groups: dry curing and wet curing. Dry cured ham and dry fermented sausages are the main representative of dry curing. In these meat products a dry cure (salt plus nitrate and/or nitrite) without any added water is applied cither on the surface of the ham or mixed with the mince for the sausage. In both cases, the products are dried and ripened for a relatively long period of time for dehydration and simultaneously for the enzymatic development of flavor (Toldra, 2002). Cooked ham and frankfurters is good representative of wet curing. In these cases a pickle injection or brine soaking is used as a vehicle for cure penetration into the product or in the case of sausages by mixing with the mince (Flores and Toldra, 1993). These products are generally cooked optionally smoked and are shorter in processing time. Flavors may be also modulated through the use of spices and/or condiments.

A great research effort on the biochemical changes and enzymes involved during meat ageing were performed during the 1970s and 1980s. However, scarce information on biochemical changes was available in other products, like cooked, dry fermented and dry cured meats (Amer, 2011). The endogenous and microbial enzyme systems were deeply studied in the 1990s, with special focus on its role in the processing and quality of these meat products (Toldra, 2002). The original ATP present in living muscle is degraded very rapidly, reaching negligible values in just a few hours. This degraded is complex and involves numerous enzymes. Main changes re observed during the first dry post-mortem. In this way intermediate degradation compounds like ADP and AMP disappear within 24h post-mortem. Other nucleotide like IMP (inosin monophosophate) shows maximum levels at $1^{st}$ day post-mortem and then slightly decreases. Inosine and hypoxanthine which are the final products of the nucleotides breakdown, increase up to $7^{th}$ day post-mortem (Batlle *et al.*, 2001).

Kurt *et al.*, (2005) conducted research work on the effects of different concentrations (0.00, 0.25, 0.50%) of either non-fat dry milk (NFDM) or whey powder (WP) on emulsion capacity (EC) and emulsion stability (ES) of beef, chicken and turkey meats. They reported when using different concentrations of WP and NFDM, 0.25% WP and 0.50% NFDM had the highest values of EC. The effect of different concentrations (0.25 and 0.50%) of WP was insignificant on ES; however, the effects of different concentrations (0.0, 0.25 and 0.50%) of NFDM were significant on ES. Casein proteins contain many proline amino acids and few disulphide bonds. They cannot be easily denatured after being heated at low temperatures. In addition, they have high electrical potential which increases solubility, thereby causing an increase in emulsion stability (ES) (Kurt *et al.*, 2005). Conversely whey proteins contain few proline amino acids and many disulphide bonds; they can contribute to gel properties which help to retain water and oil in the products. They can also absorb a higher amount of water than caseins (Smith, 1988; Ozdemir *et al.*, 1994; Isık and Gokalp, 1999).

The solubility of myofibrillar proteins increases with high ionic strength. These proteins, particularly myosin, play a significant role in the emulsification (Li-Chan *et al.*, 1985; Imm and Regenstein, 1997). Their functional properties are controlled by their species of origin as well as physicochemical factors. Arteaga and Nakai (1992) found that the white muscle fibers of turkey breast were morphologically different from muscle cells. Smith (1988) reported that fat and water holding properties of white muscle were different from red muscle. Similarly, Zorba *et al.*, (2005) found that the density and apparent yield stress of beef, turkey and chicken meat emulsions were different from each other. Lee (1985) reported comminution of muscle tissue appears to be less uniform in emulsions prepared from beef than from those prepared from chicken. Because beef contains large amounts of connective tissue, it was not completely comminuted; this was shown by the presence of many tissue pieces. Effect of lean meat source and levels of fat and soy protein on the properties of wiener type products on different meat sources, beef was more stable emulsion than pork and beef hearts in high fat (30%) and textural soy protein formulation (Sofos and Allen, 1977).

**2. 10.4.2 Textural properties of emulsion sausage**

Descriptive texture analysis occurs in several stages. All though the stages vary slightly from researcher to researcher and products to product, they occur in a logical sequence (Karen, 1993). The texture properties evaluated are hardness, cohesiveness and moisture release properties. In the frankfurter, coarseness, uniformity and denseness were also evaluated (Civille and liska, 1975). Table 2.3 shows the description or various spices. In the process of selection and consumption of food products, texture is an important factor for consumers (Moskowitz and Jacobs, 1987; Houben and Vant Hooft, 2005). Textural properties such as tenderness and juiciness are very important in consumer quality perception and may influence acceptability (Guerrero, *et al.,* 1999).

Three senses "touch, sight and hearing" may be involved in sensory assessment of texture, but in the majority of cases the sense of touch plays the most important role (Brennan, 1984). The in mouth texture is the parameter that is normally measured when evaluating dried sausage texture by sensory means, either by descriptive analysis or by hedonic methods. Textural measurements can be based on the resistance of the sample to the force of deformation such as a puncture, cutting, shear and tensiletests. Dependent variables such as adhesiveness, breaking strength, cohesiveness, fat particle size, hardness, springiness and non-dependent variables such as $a_W$, dry matter, fat content and pH maybe considered. These methods are reported simple and have the added advantage of being correlatable with sensory analysis of texture, meaning results from these methods may support those from sensory analysis (Mac 2013). When considering adhesiveness, the adhesion force can be described as a combination of an adhesive force and a cohesive force. The adhesive force is high and the cohesive force is low when food stuff is found to be sticky (Hoseney and Smewing, 1999). Dry matter content and pH of a sausage have been found to play a significant role in adhesiveness (Herrero*et al.,* 2007). Parameters such as product formulation, ionic strength, functionality of meat proteins and the content and characteristics of fat may influence the textural properties of meat products (Martín *et al.,* 2008). Spaziani *et al.,* (2009) reported using TPA with an instron UTM (Universal Testing Machine) on sample cylinders (25x 30mm diameter) for the measurement to hardness, cohesiveness and adhesiveness. Van Schalkwyk *et al.,* (2011) also reported using TPA with the Instron UTM on cylindrical cores (15x22 mm diameter) for the measurement of hardness, cohesiveness and gumminess. Rajani *et al* (2007) reported Shear force, hardness, springiness, gumminess and chewiness showed a similar trend in which prime and a significantly higher overall mean value as a result of the higher lean content. Also, he reported Adhesiveness was significantly higher for economy (0.13), where as prime and choice (0.05) and (0.04) did not differ significantly.

**Table 2.3:** Texture description of various species of meat

| Stage | Description | | | | |
|---|---|---|---|---|---|
| | Chicken breast[a] | Lamb[b] | Restructu-red beef[c] | Frankfurters[d] | Pork loins |
| **Surface** | None | Smoothness, surface moisture, fat type, fat amount of particles | None | Surface moisture, type of moisture, surface smoothness | Smoothness surface moisture, fat type, grassy (%) grassy and oily (%) fat amount of particles |
| **Partial compres-sion** | Springiness | Elasticity (springiness) | Springiness | Elasticity (springiness) | Elasticity |
| **First bite** | Initial cohesiveness, hardness, initial juiciness (moisture release) | Compressibility (cohesiveness) moisture lease, amount of fat, cohesiveness (disintegration) | Hardness, cohesivenes s, moisture release, uniformity | Hardness, cohesiveness, uniformity moisture release, denseness, coarseness, graininess | Compressibility moisture release, fat amount, grassy (%) grassy and oily (%) cohesiveness |
| **Mastica-tion (chew down and rate of malt)** | Hardness, cohesiveness of mass saliva produced particle size and shape fibrousness, chew count, bolus size bolus wetness | Chewiness, number of chews, stringiness (fibrousness) moisture release, moisture absorption, cohesiveness (of mass) fat type, fat amount, rate of breakdown, uniformity, density, connective tissue, connective tissue amount | Sample break down in two chew juiciness size of pieces Gristle cohesivenes s of mass, Webbed connective tissue, number of chews, overall gristle, overall webbed connective tissue | Chewiness. Moisture release, oiliness, moisture absorption, cohesiveness of mass, lumpy, grainy, skin, description of breakdown | Fibrousness, chewiness, number of chews, stringiness, moisture release, moisture absorption, cohesiveness, fat type, grassy (%) grassy and oily (%), fat amount, rate of breakdown, uniformity, density, connective tissue type, webbed, fibbers (%) and webbed, fibbers and gristle (%), connective tissue amount |
| **Residual** | Ease of swallowing, residual particles, tooth pack, mouth coating | Ease of swallowing, mouth coating type, mouth coating amount, the particle type, particle amount, tooth packing | Tooth packing, mouth coating | Ease of swallowing, mouth coating, oiliness, particles | Ease of swelling, mouth coating, particles and greasy (%), particles and fiber (%), mouth coating amount, the particle type, particle amount, tooth packing |

[a]Lyoun and lyoun (1990)
[b]Jeremiah (1988)
[c]Berry and civille (1986)
[d]Civille and liska (1975)
[e]Jeremiah (1990)
Source: Karen (1993)

The addition of 5% egg liquid and 5% cooked potato could have contributed to the lower TPA parameters and higher adhesiveness of the economy. Study conducted by Rajani *et al.,* (2007) indicated that the 12- day refrigerated storage of emulsions decreased the sheer force values, but caused an increase in the values of parameters such as hardness, springiness and chewiness.

Hardness is a main parameter that influences the overall acceptability of sausage products (Garcia, *et al.,* 2002). Gregg *et al.,* (1993) found a significant negative correlation ($r$=–0.86) between fat content and hardness in Bologna sausages. Cofrades *et al.,* (1997) reported that high fat frankfurters were harder than low-fat frankfurters. The reason for the discrepancy was probably due to different product formulations. Reductions of the fat content also led to an increased springiness, gumminess and chewiness, a decreased adhesiveness ($p<0.05$), but did not affect cohesiveness of cooked sausages. The improved springiness suggested that low-fat frankfurters were less brittle or more elastic (Liu *et al.,* 2008).

**2.10.5 Physiochemical characteristics of meat and meat products**

**2. 10.5.1 Fat content**

Fat and oil play vital functional and sensory roles in various food products (Youssef, 2009). Fat is one of the major food constituents which influence the organoleptical characteristic of meat products. Fat and oils play vital functional roles in various food products. Fat interact with other ingredients to develop texture, mouth feel and assist in the overall sensation of lubricity of foods (Giese, 1996). Fat quality can greatly affect lipid oxidation leading to rancid flavor and odor development in meat products. Lipid oxidation is the chemical reaction involving the abstraction of hydrogen from a fatty acid double bond and formation of a free radical (Gray, *et al.,* 1996; Kanner, 1994, Baer, 2012).

In fresh pork, fat contributes to flavor, juiciness, and texture. In addition, changes in fatty acid profile may be responsible for subjective firmness and sensory tenderness differences of pork loin chops (Leick, *et al.,* 2010). Cengiz *et al.,* (2007) reported the fat content of an emulsion type sausage has an important effect on texture and juiciness of product. Reducing the fat of emulsion-type sausages causes leaner products to become firmer, more rubbery, less juicy, and darker in color, less acceptable in term of skin formation and mouth-feel. The effect of fat on moisture contents of frankfurters was significant, but the addition of citrus fiber and soy protein concentrate did not affect the moisture content. Fat levels significantly affected protein and ash contents of frankfurters (Cengiz *et al.*, 2007). Hughes *et al.*, (1997) explained fat is known to affect the colour parameters of cooked meat products. Similar result found by Crehan, *et al.*, (2000) Reducing the fat content from 30% to 5% caused a significant decrease in the lightness of frankfurters.

### 2. 10.5.2 Protein content

Meat proteins are of three types: the first sacroplasmic proteins which are often referred to as water soluble protein. The second, myofibrillar protein, which also known as contractile proteins by virtue of the key role they play in muscle contraction and locomotion in the living animals. The third type is connective tissue proteins which function as a supporting framework for the dividing body and thus serve in numerous and varied functions. Muscle proteins, accounting for approximately 200 g $kg^{-1}$ muscle, are of great significance for the maintenance of meat product structure. Myofibrillar proteins, the main component of muscle proteins (ca 550–600 g $kg^{-1}$ total proteins), can be further subdivided into three classes, i.e. structural proteins (myosin, actin, etc.), which build up the main structure of muscle, regulatory proteins (troponin, etc.) and cytoskeletal proteins (titin, nebulin, desmin, etc.), which support the whole myofibrillar structure. However, sarcoplasmic proteins mainly include enzymes involved in energy metabolism. Collagen is the main component of connective tissue proteins and is located extracellularly. Previous studies have revealed that sarcoplasmic proteins aggregate between 40 and 60 ° C[9] and that myosin and actin denature at temperatures lower than 50° C and higher than 65° C respectively (Huang, *et al.*, 2010; Xia, *et al* 2008).

### 2. 10.5.3 pH

Generally, meat from freshly killed animals has an average pH of 6.8, which falls rapidly to 5.4-5.6 (ultimate pH) in duration of 48 hours postmortem. Depending on the storage conditions, status of meat chilling and the degree of bacterial contamination the pH may remain constant for a while or begin to rise gradually due to autolysis and the growth of bacteria. When meat reaches a pH of 6.4, decomposition may set in and at 6.8 and above the signs of decomposition viz., changes in color, odor and texture and may become apparent. Meat with pH greater than 5.8 may be more able to spoilage and result in decreasing the shelf life (Price *et al.,* 1987). The level of pH attained may depend on several factors, such s kind of animal, level of nutrition, physiological status before death degree of stress prior to slaughter, amount of muscle glycogen, a heredity type of muscle and temperature during the post mortem glycolysis (Ahmad, 2005). *Rajani et al.,* (2007) reported the quality of chicken emulsion, overall increased in pH was less than 0.1 unit during the 12- day storage period but showed a progressive increase.

The ultimate pH value of raw buffalo meat and time to attain the pH value depends upon many factors, namely holding temperature, type of muscle i.e. protein of the carcass and glycogen content of the muscle (Ahmad, 2005). The pH value of buffalo meat emulsion sausage highly significant ($p<0.01$) increase which increase in level of topica, and the pH emulsion increased the level of inclusion upon cooking which might attributed to the fact in cooking range 55-80°C (Ponsingh, 2011). The estimate of pH of meat is of value in determining durability while judging the suitability of borderline carcass meat for further utility. Meat of alkaline nature will exhibit faster rate of microbial spoilage than of the acid. The pH of meat has a significant relationship to eating quality. The low ultimate pH generally increases tenderness, which is positively correlated to WHC (Govindarajulu, 2002).

**2. 10.5.4 TBA number**

Thiobarbituric acid (TBA) number is important relevant characteristics of meat product that indicate the oxidation state and later on stage rancidity of the product (Ahmad and Amer, 2013). Thiobarbituric acid (TBA) value measures secondary lipid oxidation products, which were also responsible for the rancid taste developed during storage (Rossell, 1989). TBA test is used as an index for measuring oxidative rancidity (malonaldehyde formation), which takes place in meat. Furthermore, TBA-test is a sensitive test for the decomposition products of highly unsaturated fatty acids which do not appear in peroxide determination (Melton, 1983). Warmed over flavour (WOF) in meat was first recognized by time and Watt (1985), is a form of oxidative rancidity that then develops within a few days in contrast to common rancidity that requires two months to develop fully during freezer storage

(Pearson *et al.*, 1977: Igene *et al.*, 1979). Although WOF can develop in fresh meat, it most commonly occurs in meats that are cooked or in which membranes are broken down by a process such as restructuring or grinding (Ahmad, 2005).

TBA measurements have been frequently found to give useful correlation with sensory scores, in looking at the development of warm over flavour in cooked meats (Poste *et al.*, 1986).TBA test can be used for oxidation in muscle foods, although the test should be accompanied frequently by corresponding evaluation with a trained sensory panel in studies of WOF in meat (Igene *et al.*, 1979). Thiobarbituric acid (TBA) value less than 1–2 mg malonaldehyde per kg meat is regarded as the acceptable level in meat products (Greene and Cumuze, 1981). The TBA values were below the critical values that indicate spoilage of meat products, particularly up to day 9 of refrigerated storage. This could be due to the addition of phosphate, which was reported to produce antioxidant effects in meat systems (Salahuddin *et al.*, 1989), and also because of the addition of a mixture of spices and condiment ingredients, which have variable antioxidant properties (Coggins, 2001). Tarladgis *et al.*, (1960) reported that TBA numbers were highly correlated with sensory scores of trained panelists for rancid odors in ground pork. There is a correlation between sensory attribute and TBA number of meat and meat products. This researcher advocated that threshold range of TBA number for detecting off odour in pork was approx. 0.5-1.0. While in cooked beef the values were in the range of 0.6-2.0. It has relation between TBA numbers and assessed oxidized flavor in cooked beef (Greene *et al.*, 1982).

### 2. 10.5.5 Water Holding Capacity

Water holding capacity (WHC) is the ratio of moisture retained in the sample of the initial moisture content, so the higher percentage indicates release of less moisture. Water holding capacity is a measure of how much water is bound in the uncooked state. WHC capacity is a general term used to describe the extent of water held or bound by meat products once cooked. It includes the more specific term used in literature such as cooking yield, cooking loss and water binding value (Trout *et al.*, 1983). A high WHC in the lean meat is a decisive factor for producing a high quality sausage (Hamm, 1970). It is also prevents rendering out of fat and results in lower losses during smoking, cooking, storage and canning of the sausages. Grinding of meat increase Water holding capacity by enhancing the number of polar groups available for binding with the water molecule and the water is bound better when added after the meat ground (Hamm, 1970).

In sausage WHC significantly increased with increase of fat replace ingredients. Water holding capacity is a measure of how much water is bound in the uncooked state. Water holding capacity capacity is a general term used to describe the extent of water held or bound by meat products once cooked. It includes the more specific term used in literature such as cooking yield, cooking loss and water binding value (Trout *et al.,* 1983). Water holding capacity is directly related to eating quality and influenced by pre-slaughter, slaughter and post slaughter techniques. The water in muscle available in two forms bond and free water, the former amounting to less than 5% is directly bound to the hydrophilic groups of proteins and the latter is responsible for most of the changes observed in water holding capacity of meat. A diminution of in vivo WHC is manifested by exudation of fluid termed as weep in meat that has not been frozen, as drip in thawed meat and as shrink in cooked meat, derived from both aqueous and fatty sources. The techniques of slaughter and post slaughter seem to influence WHC. The WHC of fresh meat is high and drop gradually within a few hours, reaching the minimum in 24 to 48 h but slowly increasing during further storage (Ahmad, 2005).

The meat at pH 7 will have a high WHC, the muscle absorbing and retaining approximately equal volume of water, at pH 6 less than 50%and at pH 5 (isoelectric point) about 25%. Consequent to the death of an animal the loss of Water holding capacity is inevitable. The extent of the fall of the post mortem pH will be affecting the WHC, higher the ultimate pH, the greater is the WHC. It is therefore clear that the meat possessing high ultimate pH, chilled rapidly before the one set of rigor mortis and undergoing retarded post mortem glycolysis, will enhance WHC. Much interest has been shown to diminishing weep or drip in meat used for making sausages, emulsion, retail cuts. Sausages and comminuted meats are more liable to exude fluid due to destruction of a structure during processing there by lowering the ability to retain fluid. It is important to consider the behavior of water added to meat to affect the overall Water holding capacity of the mix. The normal ratio of water to meat is about 2:1 (Ahmad, 2005).

### 2. 10.5.6 Extract Release Volume

Evaluation of the extract release volume (ERV) phenomenon is a rapid test for determining spoilage in meat. Extract release volume appears to have considerable possibilities for assessing the spoilage of beef (Jay and Konton 1964). It also has a highly significant correlation with Water holding capacity. The procedure is based on measuring the volume of the aqueous filtrate released from slurry of meat in fixed time. The ERV decreases as spoilage progresses and no filtrate is obtained from putrid meat (Singhal *et al.,* 1997). The extract release volume method reveals two aspects of the spoilage mechanism. First, low temperature meat spoilage occurs in the absence of any significant breakdown of primary proteins. The second aspect of meat spoilage revealed by ERV is the increase in hydration capacity of meat proteins by some as-yet unknown mechanism (Jay *et al.,* 2005), although amino sugar complexes produced by the spoilage biota have been shown to play role (Shelef and Jay1969a). With the microbially spoiled meats the highest degree of correlation occurred between meat swelling (SW) and extract release volume, followed by extract release volume and water holding capacity, meat swelling and viscosity, viscosity and extract release volume and meat swelling and water holding capacity. These findings indicate that ERV and SW are quite similar and equally reliable in determining meat microbial qualify while the relationships between viscosity and ERV, and viscosity and WHC were of lower orders of significance (Shelef and Jay1969b). Various relationships between the extract-release volume and the protein and fat contents of fresh beef are studied in relation to theoretical and other considerations. Inter-relationships between the pH, total volatile nitrogen and the extract-release volume of stored beef samples confirm that the drop in the filtrate volume as spoilage advances is due to changes in the protein. The correlations between the ERV and the pH values take into account the changes which occur in both the 'reverse'prerigor and post-rigor stages (Pearson, 1968).

### 2.11 Incorporation of whey protein products

Whey is defined as the watery component removed after the setting of the curd in cheese manufacture. The two main types of whey are sweet whey and acid whey. Acid whey is produced from cheese that is directly acidified with a mineral acid or lactic acid, whereas sweet whey is the result of starter culture acidification of cheese

(Bordenave *et al.*, 2005). Acid whey has atitratable acidity of 0.4%, a pH less than 5.0 and is derived from fresh acid cheeses. Whey protein (WP) is the water phase of milk once casein has been removed. Its flavours is generally equivalent to that of meat and it also matches the light colour of poultry meat well (Feiner, 2006).

Sweet whey generally has a titratable acidity between 0.1- 0.2 percent, a pH of 5.8-6.6 and is derived from rennet coagulated cheeses like Cheddar. Because of the different treatments, sweet and acid whey has different mineral, protein, and lactose properties as well as different functional properties (Bordenave *et al.*, 2005).

Whey contains approximate 65g dry matter per kilogram which consists of 50g lactose, 6g protein, 6g ash, 2g non-protein nitrogen and 0.5g fat. The protein consists of 50% β- lactoglobulin, 25% ά-lactalbumin, and 25% of other proteins including immunoglobulins, proteose-peptone, bovine serum albumin and β-casein (Varnametal., 1994). Whey is made up of a number of proteins including beta-lactoglobulin, alpha- lactalbumin, Bovine Serum Albumin (BSA) and Glycomacropeptide (GMP). Whey proteins (WPs) are a nutritious ingredient that is manipulated to impart texture in many foods (Firebaugh, 2004). Whey protein is pure, natural and high quality protein. Whey proteins developed from milk during the process of cheese. WPs are globular molecules with a substantial content of a-helix motifs, in which the acidic and hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains (Evans, 1982). The actual concentrations of whey proteins depend on the type of whey (acid or sweet), the source of milk (bovine, caprine or ovine), the time of the year, the type of feed, the stage of lactation and the quality of processing (Pintado *et al.*, 2001).

The WP has chemical and physicochemical properties including b-lactoglobulin, a-lactalbumin, immunoglobulins, bovine serum albumin, bovine lactoferrin and lactoperoxidase with other minor components (Madureira *et al.* 2007). Whey protein conformation and physicochemical properties depend on a number of factors, including pH, solute concentration, ionic strength, temperature, and others (Cayot and Lorient, 1997; Kinsella and Whitehead, 1989). The pH of the dispersing medium affects the net charge of proteins. Thus, due to the polar nature of water, a net negative or positive charge on protein molecules makes them more water soluble than if the net charge is minimal, i.e. at the isoelectric point (Kinsella and Whitehead, 1989). Salt concentration also affects the physicochemical properties of whey proteins. Salt concentrations up to 0.1M enhance whey protein solubility, but concentrations beyond 0.15M can result in salting out of the protein (Kinsella and Whitehead, 1989; Zayas 1997). Many researcher reported the main biological activities of WPs such as Prevention of cancer (breast and intestinal cancer, chemically induced cancer), increment of gluthatione levels (increase of tumour cell vulnerability, Treatment of HIV patients), antimicrobial activities and increment of satiety response (increment in plasma amino acids, cholecystokinin and glucagon-like peptide) ( Gill *et al.,* 2000; Badger *et al.,* 2001; Maclntosh *et al.,* 1995; Hakkak *et al.,* 2000; Rowland *et al.,*2001; Parodi, 1988; Micke *et al.,*2001; Micke *et al.,* 2002; Clare *et al.,* 2003; Hall *et al.,* 2003). Whey proteins are surface-active globular proteins and can be adsorbed at the fat/water interface where they unfold, and potentially can help stabilize the fat globules within a food matrix (Huffman, 1996; Sun Gunasekaran and Richards, 2007). Such functional properties make whey proteins a useful additive in the production of meat emulsions and therefore whey is commonly used to improve emulsification, water binding and texture (Holland, 1984; Shie, 2004).Whey proteins having excellent surface-active properties that allow them to re-orient and reduce interfacial tension with increased opportunity for fat- protein interacting (Lucca and Tapper 1994). Whey proteins have the ability to form thermally induced gels when heated above 70 °C, positively affect meat products' stability and texture (Swaisgood, 1982; Langley and Green, 1989).

Proteins in whey have low molecular weight and are able to expose hydrophobic groups when partially unfolded. Therefore, WPs rapidly migrate and adsorb onto in air-water interface, reducing surface tension, and allowing them to form stable foams (Philips *et al.,* 1990). This property is usually identified with the foaming ability of WP products in an aqueous solution (de Wit, 1998). Milk proteins are good moisture binder when used in meat processing, although they have lower emulsifying capacity on a soluble protein basis (Mittal and Usborn 1985; Zorba *et al.* 1995). Additions of dairy ingredients have been used as fillers and binders in comminuted meat products to improve texture and sensory properties and minimize cooking loss (Hung and Zayas, 1992). Addition of dairy ingredients significantly increased water holding capacity and emulsion stability and also added dairy ingredients has lower cooking (Meltem and Eylem, 2004). Dairy proteins have been incorporated as water and fat binders and have the potential to modify the textural characteristics of low fat comminuted meat products (Comer *et al.*, 1986). Proteins are frequently added to meat products for a variety of reason. They can stabilized emulsions given that solublized proteins have hydrophilic (water loving) and lipophilic (fat loving) groups within the molecule and therfore they act as emulsifier, holding two mixable phases together during heat treatment. Protein also bind water on a molecular basis owing to hydrogen bonds within the solublized protein itsself and therefore proteins also help to increase firmness of products (Feiner, 2006). Some proteins even enhance the flavour in finished products and protein is also occasionally added to meat products to raise the total level of protein to fulfil legal requirements (Feiner, 2006).

Whey proteins are very effective emulsifiers of fat and oil. Their form of emulsion can use to replace meat proteins, chemical emulsifier and non meat protein products such as soy protein. Additionally, the bound fat in whey protein products is relatively high in phospholipids adding to the emulsification capacity of any given whey ingredient (Keaton, 1999). When using whey products to increase finished product yields, care is needed to consider the potential dilution of added spices and seasonings. Slight increases in spices and seasonings may be required to return the resulting extended meat product to the best flavor balance (Keaton, 1999).

Whey proteins are generally used in infant formulas and sports food as a nutritional supplement (Jooyandeh, 2011; Ramos *et al.*, 2012). Whey protein has been used in confectionery, bakery and ice cream products, health foods and sport, and also used as food ingredients in the cause of their smooth functional and nutritional properties. Their desirable functional properties such as solubility, foaming, emulsification, heat-induced gelation and coagulation, water binding capacity and retention, dispersability, viscosity and turbidity have been primarily revealed and utilized in food systems (Foegeding and Davis, 2011; Firebaugh and Daubert, 2005). The properties of whey based protein products are mainly dependent on the processing technology. Several different treatments including heat treatments and membrane fractionation techniques significantly influence their properties and consequently their possible uses (Almécija *et al.*, 2007). An important of WPs is their success as emulsifiers in food systems.

Determining their emulsification properties are protein concentration, pH, ionic strength, concentration of calcium and lactose, the processing history, and the storage conditions (Mccrae *et al.*, 1999). The WPs allow the fat globules as structural elements to good emulsifying properties. Moreover, the well-known heat-induced interactions between WPs and casein micelles make milk an interesting base for all kinds of textured products with high nutritional values (de Wit, 1998). Yetim *et al.*, (2001) reported emulsion stability rate was significantly increased by the liquid whey addition to the formulation. A slight increase in ash content and pH value was observed with the liquid whey addition. Even 100% whey replacement did not produce any adverse effect in cooked sausage sensory properties. Whey protein showed excellent nutritional and functional properties in low fat meat products (Perez-Gago Krochta 2001). Whey proteins improved emulsion stability, provided better color propertied and resulted in lower chewiness and elasticity, but higher brittleness and hardness in frankfurter type sausages (Yetmin *et al.*, 2001). When preheated whey protein was used in poultry raw and cooked meat batter it resulted increase water holding capacity and improves the rheological properties and reduce cooking loose (Hongsprabhas and Barbut, 1999). Addition of whey protein did not affect in fat and protein content meatballs, addition of whey powder beneficial in improving cooking characteristics at each fat level (Melton 2006).

Onwulata *et al.*, (2003) studied the functional of texturised WPs by extruding three different types of WP including WPC, WPI, and whey albumin at 38% moisture content at different cooking temperatures. They found that varying temperatures in the extruder demonstrated different degrees of WP denaturation, which might be useful for different products. Table 2.4 shows the functionality of whey products in processed meats. Whey protein can to replace or supplemented the functionality of meat proteins in processed meats. Some of this functionality (emulsification, water binding, and gelation) can come from the highly functional non-casein whey proteins. This can result in improved emulsion stability, slicing characteristics, cook yields (improved water binding), flavor, and reduced costs. The use of whey proteins can also be considered as partial replacement of meat proteins, partial or total replacement of soy protein products and other non-meat binders/fillers, modified starches and hydrocolloid gums (alginates, gum arabic and others) in processed meats. The result is processed meats with improved functional performance, flavor and cook yields, at significantly reduced costs (Keaton, 1999).

Kurt *et al.,* (2005) reported the addition of whey protein increased protein concentration and pH and emulsion capacity beef, turkey and chicken meats. Addition of WP in meat increased the solubility of proteins (Zobra *et al.,* 1995). The WP addition increased emulsion solubility; this result can be explained by the fact that whey proteins have a high capacity to bind water (Kocak *et al.,* 1994). Whey proteins aid in improving firmness, texture and retention of moisture during processing and cooking. Reduction of purge (moisture loss) in vacuum-packed meats is also a consideration. This important function can result in significant yield improvements. Whey proteins add positive textural attributes: chew, bite, firmness, and smoothness, non-coarse, non-grainy, non-gritty mouth-feel improvements. WPs are very efficient emulsifiers of fat and oil. They form stable emulsions easily and can be used to totally, or partially, replace chemical emulsifiers, meat proteins, and other non-meat proteins such as soy protein products. Additionally, the bound fat in whey protein products is relatively high in phospholipids adding to the emulsification capacity of any given whey ingredient whey product performance in processed meats is the ability to control mix ingredient costs and improve cook yields (Keaton, 1999).

**Table 2.4**: Functionality of whey products in processed meats

| **Function** | **Specific impact** |
|---|---|
| Solubility | Smooth texture at most use levels<br>Creamy textures at high usage rates<br>Reduced "gritty," "powdery" taste |
| Water binding | Binds and entraps water: improves cook yield<br>Provides body, texture<br>Improves sliceability |
| Viscosity | Thickening<br>Enhances body, texture |
| Gelation | Forms gel during heat processing |

| | |
|---|---|
| | Animal/fat replacement possible |
| Emulsification | Forms stable, fat/oil emulsions<br>Prevents oiling-off and ''fat caps''<br>Meat protein replacement |
| Foaming | Forms stable film<br>Provides structure |
| Browning | Enhances Maillard, non-enzymatic browning<br>Adds color, visual appeal |
| Flavor, aroma | Have little or no flavor of their own<br>Compatible with cooked meat flavors<br>Compatible with spice/seasoning blends |
| Nutrition | Superior amino-acid profile<br>Can serve as a source of calcium for enrichment |

Source: (Keaton, 1999)

In the processing technology whey products have different type products, namely whey protein concentrates with 25-89% of protein concentration, whey protein isolate with range of protein concentration 90-95% and whey protein hydrolyzed with variable range of protein concentration. Fig 2.2 shows the different whey proteins manufactured from skim.

### 2.11.1 Whey Powder

Whey separated from the curd after the coagulation of milk during the manufacture of cheese. Approximately 9 liters of whey can be produced from 10 liters of milk for every kilogram of cheese produced. This watery residue, or serum, is a huge reservoir of high-value milk protein. Because whey contains more than 90 percent water, it is essential to remove some or most of the water. This enables the productions of various types of whey powder which are sought after for their unique functional properties. The most common types of whey powder are demineralized whey powder and lactose and delactosed whey powder.

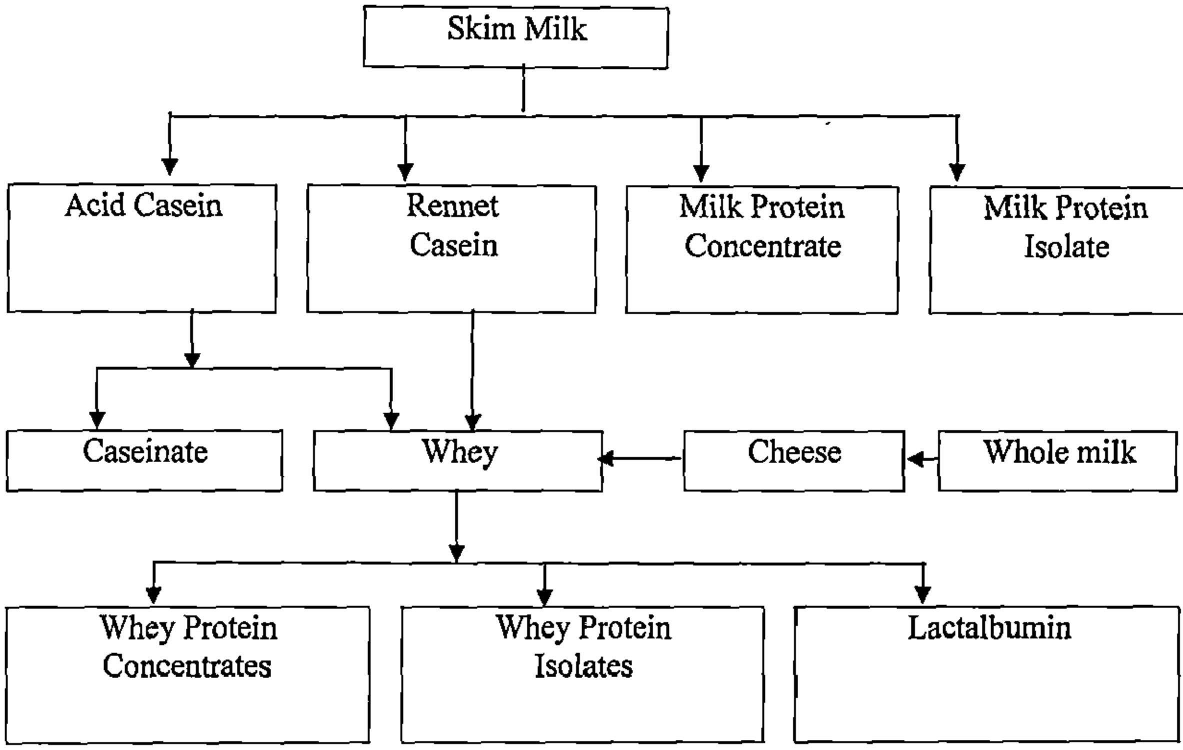



**Figure 2.2:** Whey protein manufactured from skim or whole milk (Huffman and Harper, 1999)

Whey powder consists primarily of carbohydrate (lactose), protein (several different whey proteins, mainly lactalbumins and globulins), various minerals and vitamins. The composition of whey powder total solids 96-97%, Lactose 70-75%, total protein (N x 6.38), 10-13% minerals (ash 7-12%, calcium 4500 mg/kg) and vitamins (Thiamine 0.4-0.6 mg, Riboflavin 2.3-2.5 mg and Pyridoxine 0.4-0.6 mg) (Canadian Dairy Commission, 2011; Huffman and Harper, 1999). Table 2.5 shows the typical compositions of whey products and Table 2.6 shows the nutritional information of whey protein powder.

Sweet whey powder is one of the whey powder produced by drying fresh whey from which the milk fat has been removed during the preparation of Cheddar, Mozzarella, Swiss or other cheeses manufactured principally with rennet type enzymes (casein-coagulating enzyme preparations). It contains all the constituents, except water, in the same relative proportion as in liquid whey. Its pH in a 10 percent solution should be greater than 5.6. This type of whey powder represents the majority of the Canadian production. Acid whey powder is another type of whey powder produced by drying fresh whey obtained from the Cottage, Ricotta or other fresh cheeses manufactured principally by acid coagulation (the pH of the milk is lowered by microbial fermentation process in which some of the lactose is converted to lactic acid). It is similar to sweet whey powder in composition except for its lower lactose content and its higher titratable acidity which makes its flavor, slightly acidic compared to normal whey flavor found in sweet whey powder. Its pH in a 10 percent solution should be lower than 5.1. Demineralized whey powder is tertiary type of whey powder produced from whey by selective removal of most (70-90 percent) of the minerals. Delactosed whey powder is fourth number of whey powder produced from whey by crystallizing a majority of the lactose out and recovering the primary substance. Whey powders shall contain less than 5 mg/kg of copper, 1 mg/kg of lead and 20 mg/kg of iron. They are exempted from Salmonella, Listeria, coagulase-negative Staphylococcus and show a maximum Standard Plate Count of 50,000 colony-forming units/gram (cfu/g) (Canadian Dairy Commission, 2011).

**Table 2.5**: Typical compositions of whey products

| | Whey powder | Whey protein concentrate 34 | Whey protein concentrate 80 | Lactalbumin | Whey protein isolate |
|---|---|---|---|---|---|
| Protein % | 13 | 34 | 80 | 90 | 92 |
| Lactose % | 75 | 53 | 6 | 0.5 | 1 |
| Ash % | 8 | 7 | 3 | 0.5 | 2 |
| Fat % | 1 | 3 | 7 | 4 | 1 |
| Moisture % | 3 | 3 | 4 | 4 | 4 |

Source: Huffman and Harper, 1999

Whey powder acts as a binder and extender for many food products, such as beef preparations, chili con carne, poultry, sausage, soups and stews. Usage level is limited to 3.5 percent for sausage and 8 percent for chili con carne, pork or beef with BBQ sauce (Barbecue sauce). It is widely used in bakery products, dry mixes, process cheese foods and spreads, frozen desserts, sauces, meat emulsions, salad dressings, confections, gravies, snack foods and beverages. Demineralized whey powder as well as delactosed whey powder is used in infant foods, diet food formulations, prepared dry mixes and for food products in which mineral profile and concentration are critical attributes (Canadian Dairy Commission, 2011).

**Table 2.6:** Nutritional information of whey protein powder

| **Ingredient** | **Amount per 100g** |
|---|---|
| Energy | 280 kcal |
| Protein | 65 g |
| Carbohydrate | 5 g |
| Fat | 0 g |
| Vitamin A | 120 mcg |
| Vitamin C | 20 mg |
| Vitamin E | 12 mg |
| Vitamin $B_1$ | 250 mcg |
| Vitamin $B_2$ | 335 mcg |
| Vitamin $B_6$ | 500 mcg |
| Folic Acid | 20 mcg |
| Vitamin $B_{12}$ | 0.25 mcg |
| Calcium | 75 mg |
| Iron | 5 mg |
| Phosphorus | 35 mg |

Source: Venky's nutrition (2012)

#### 2.11.1.1 Functional Properties whey powder

Nutritional and functional characteristics of whey proteins are related to the structure and biological functions of these proteins. Native whey proteins (globulins and lactalbumins), as 11 percent of the constituents of whey powders, possess good functional properties related to solubility, foaming, emulsion formation and gelling. The high content of lactose plays an important role in flavor, aroma and browning reactions (Canadian Dairy Commission, 2011).

### 2.11.2 Whey protein concentrates

Whey protein concentrate is the substance obtained by the removal of sufficient non protein constituents from whey so that the finished dry product contains 25 to 89.9% protein. It should be not more than 10% milk fat, 5% moisture and pH exceed 7.0 (USDA, 2003), Ash content is set at 2 - 15%, while lactose content is a maximum of 60% and the limit on heavy metals is 10ppm (FDA, 1999) (Zevchak, 2007). Whey protein concentrates are commonly manufactured using filtration techniques to concentrate protein based on molecular weight differences. During ultrafiltration, lactose, salts, and other low molecular weight materials pass through a membrane in the permeate, while higher molecular weight components such as protein are concentrated in the retentive. The concentrated protein solution is then sprayed dried to form a WPC powder, which generally ranges in protein concentration from 34 to 89%. High protein whey protein concentrates (>80% protein) are used in a wide range of foods, including meats, bakery, surimi, and confections, because of their ability to impart viscosity, hold water, gel, foam, and emulsify (Huffman, 1996).

Whey protein concentrates contain residual lipid despite attempts by producers to remove as much lipid as is possible from the whey. Removal of residual lipid from whey has been shown to increase ultrafiltration flux and to improve whey protein concentrate functionality. It has been shown that the lipid content of whey protein concentrates tends to increase as the protein content also increases. Residual lipid has long been recognized as being detrimental to the quality of whey protein concentrates with particular attention to the foaming and flavor qualities of the product. Table 2.7 shows the physicochemical and microbiological characteristics of whey protein concentrate 70%.

Modified whey protein concentrate is an important functional ingredient having wide range of application in food products. An important functional properties of the whey protein are hydrophilic, swelling and water retention capacity and its ability to absorb and bind water is useful in connection with frozen dough's which are mixed, formed and then held in frozen storage for some length of time before being thawed, proofed and baked (Asghar *et al.,* 2009). Whey proteins contain many hydrophilic groups that are exposed upon heating and react with water, thus increasing the meats water holding capacity. Upon cooling, the proteins restructure entrapping water and preventing moisture loss (Dybling and Smith, 1991).Whey protein concentrate incorporated with wheat flours of protein contents 9.2, 12.7 and 14.2% for making frozen dough in storage in freezer at -40 F, were affected a significant decrease in the values of hardness, cohesiveness, gumminess and springiness were observed with its addition in dough samples (Asghar *et al.,* 2009). Developed snack product with ingredient if extruding a dry mix comprising 2 parts of

WPC 80 and 1 part of corn starch at a rate of 25 g/min and adding 0.1 M NaOH solution at a rate of 11 g/min at 145 - 147°C, the result was crunchy and even with small cell size (Walsh, 2003).

Andres *et al.*, (2006) reported chicken sausage with a total lipid content in the range of (0.22%, to 6.09%), with increasing WPC decrease the sausage hardness and cohesive was obtained. Low-fat chicken sausages incorporated with xanthan and guargums and WPC had good acceptable sensory scores and functional properties (Andres *et al.*, 2006). Exhaustively washed chicken breast muscle improved by the addition of WPC in the emulsion stability heated cream layers (Imm *et al.*, 1998). The addition of 2% of WPC in knockwurst sausage, the sausage had higher stability than with 0.00, 1.75, or 3.75% addition of WPC (Ensor *et al.*, 1987). Whey protein concentrates (WPCs) have the ability to improve the water and fat binding properties, as well as enhancing sensory and textural attributes of meat products (Chen and Trout, 1991; El-Magoli, *et al.*, 1996; Hughes, *et al.*, 1998; Szerman *et al.*, 2007). In another study Szerman *et al.*, (2008) reported that the addition of WPC was effective to partially replace the sodium chloride in whole beef muscles, producing comparable yields and improving sensory attributes.

El-Magoli *et al.*, (1996) explained that addition of whey protein concentrate would increase fat binding in the meat system even at lower fat levels (10%). Addition of high gelling 35% WPC increased purge losses and did not significantly influence cook loss or water holding capacity of low fat cooked sausages (Lyons *et al.*, 1999). The addition of WPC to meat products increases the WHC, with the result of reduction in cooking weight loss. This addition also improves sensory quality and enhances nutritional values of meat products (Thomsen, 1996). The sensory results showed that no any identified differences in taste or texture between burgers made using 40% base texturized WPs and 100% beef (Hale *et al.*, 2002). Incorporation of whey protein concentrates at the rate of 1-4% in pork patties improved the cooking yield compared to a non-formulated control of 10% fat. Also the addition of the level of WPC improved fat retention (El-Magoli, *et al.*, 1995).

Developed meat extender for beef patties by extruding 2 parts of WPC and 1 part of corn starch using water, 0.1 N HCL, or 0.2 M NaOH as the liquid. The sensory results showed that no any identified differences in taste or texture between burgers made using 40% base texturized WPs and 100% beef (Hale *et al.,* 2002). Incorporation of whey protein concentrates at the rate of 1-4% in pork patties improved the cooking yield compared to a non-formulated control of 10% fat. Also the addition of the level of WPC improved fat retention (El-Magoli, *et al.,* 1995).

**Table 2.7:** Physicochemical and microbiological characteristics of whey protein concentrate

| **Physicochemical characteristics** | |
|---|---|
| Color | White |
| Taste | Bland |
| Sediment, ADPI* Disc | B |
| pH (10% Ww/v Solution) | 6.62 |
| Bulk density (gm/ml) | 0.43 |
| Moisture % | 3.20 |
| Fat % | 3.66 |
| Protein % ODB | 70.02 |
| Total minerals % | 3.04 |
| **Microbiological characteristics** | |
| Total plate count per gm | 2100 |
| Coliforms/0.1gm | -ve |
| Salmonella/100gm | -ve |
| Yeast and mold/ gm | <10 |

Source: Mahan protein limited (2012); * American Dairy Products Institute

### 2.11.3 Whey protein isolates

Whey protein isolates (WPI) contain >90% protein and can be prepared by either microfiltration membrane process. The process by which WPI itself is manufactured will affect the overall composition of individual proteins, which influences functional properties such as gelatin, foaming, and emulsification. Differences in protein composition also give rise to somewhat different functionality between WPC and WPI. Manufacturing of whey protein isolate should use either microfiltration or an ion exchange to purify the whey protein. Whey protein can act like an acid or base depending on the pH of the medium. Whey proteins have a net positive charge at pH values below their isoelectric point and a net negative charge above their isoelectric point (Varnam and Sutherland, 1994). The pH can be adjusted so that the proteins will be absorbed onto a proper ion exchange that has a pore size and surface suitable for the recovery of proteins from dilute solutions (Varnam and Sutherland, 1994). Supplement facts of whey protein isolate shows table 2.8.

For a sports drink beverage with high acid and low pH it should use whey protein isolate which can withstand high heat and low pH, while remaining clear in the liquid (Dahm, 2005). pH must be 3.0 or less for a WPI beverage to remain clear after thermal processing at 88° C for 120 seconds (Etzel, 2004).

Preheating a whey protein isolate before addition to meat products improved water holding capacity, cooked yield and rheological properties of raw and cooked poultry breast meat batters (Hongsprabhas *et al.,* 1999). Whey protein concentrate, whey protein isolate and simple whey powder can improve the emulsion stability of frankfurter sausage (Yetim *et al.*, 2001).

**2.12 Effect of incorporation non meat ingredient in sausage**

A few decades non meat ingredients have been used in meat products to improve the quality of the products and reduce the cost of products. These ingredients are spices, dietary fibers, vegetable protein and probiotics. These additives are able to increase nutritional value, consumer acceptability and benefits to human health's. Emulsion stability rate was significantly ($p<0.05$) increased by the liquid whey addition to the formulation and a slight increase in ash content and pH (Yetim *et al.*, 2001). Even 100% whey replacement did not produce any adverse effect in cooked sausage sensory properties. Milk proteins are good moisture binder when used in meat processing, although they are a lower emulsifying capacity on a soluble protein basis (Mittal and Usborn 1985; Zorba *et al.* 1995).

**Table 2.8**: Supplement facts of whey protein isolates

| **Amount per serving** | **Amount per 100g** |
|---|---|
| Calories | 431 |
| Total fat | 10g |
| Cholesterol | 12.5mg |
| Sodium | 194mg |
| Potassium | 417mg |
| Total carbohydrate | 14g |

| | |
|---|---|
| Protein | 69g |
| Vitamin A | 28I/U |
| Calcium | 417mg |
| Iron | 3mg |

Source: Gaspari Nutrition, USA (2012)

Additions of dairy ingredients have been used as fillers and binders in comminuted meat products to improve texture and sensory properties and minimize cooking loss (Hung and Zayas, 1992). Addition of dairy ingredients significantly increased water holding capacity and emulsion stability and also added dairy ingredients has lower cooking (Meltem and Eylem 2004). Skim milk powder is widely used as neutral filler with good water binding effect in comminuted meat products, but lactose may cause discoloration of meat products because of maillard reactions with proteins (Ellekjaer *et al.,* 1996). Dairy proteins have been incorporated as water and fat binders and have the potential to modify the textural characteristics of low fat comminuted meat products (Comer *et al.,* 1986). Additions of milk protein as dry ingredients reported have effects on the texture of comminuted meat products and resulting change from springy at lower protein concentrations in cakey and dry higher concentrations (Comer *et al.,* 1988; Baard seth *et al.,* 1992). Whey protein showed excellent nutritional and functional properties in low fat meat products (Perez-Gago Krochta, 2001). Whey proteins improved emulsion stability, provided better color propertied and resulted in lower chewiness and elasticity, but higher brittleness and hardness in frankfurter type sausages (Yetmin *et al.,* 2001). Pre- heated whey protein isolate formed gel at low temperature in the presence of added salt (Hongsprabhas and Barbut, 1997). Addition of whey protein did not affect in fat and protein content meatballs, addition of whey powder beneficial in improving cooking characteristics at each fat level (Melton, 2006). Ander (2006) conducted studies when the gums and whey protein concentrate applied in chicken sausage within the range, weight losses were low (2-4%) and no significant effect, also fat content showed a marked effect on lightness and redness and textural parameter resilience. Ander (2006) described by increasing gums and WPC decreased sausage hardness, while increasing WPC or gum concentrate a more cohesive and less granular matrix was obtained in chicken sausage. The addition of Beta-lactoglubin fractions reduced the cook loss ($p<0.001$)

and reduced water holding capacity ($p<0.001$), also increased the TPA value of hardness and decreased the springiness with the lowest mineral level in frankfurters sausage (Hayes *et al.,* 2005). The growth of aerobic bacteria and Listeria monocytogenes were inhibited and moisture loss was decreased by 31.3% in sausages with whey protein coating (Shon and Chin, 2008). Soy proteins are commonly used in processed meat products for their factional properties and low cost compares lean meat (Chin *et al.,* 1999). Incorporation of 2% soy protein isolate level increased moisture content and cooking yield while decrease purge loss of the light pork sausage were less red and more yellow the addition of SPI was not less than 1.5% level (Akesowan, 2008). Ahmad *et al.,* (2010) reported incorporation of soy protein brought a considerable change in physic-chemical, microbiological, sensory and textural characteristics of law fat emulsion sausage. Soy protein has been incorporated processed meat products for water binding capacity and fat binding ability, enhancement of the emulsion stability and increasing yield (Chin *et al.,* 2000). Chin *et al.,* (1999) reported soy protein isolate resulted in a softer texture of low fat bologna and did not affect other chemical parameter. Feng *et al.,* (2002) described heat and enzyme hydrolyzed soy proteins effected texture properties differently, the 1$^{st}$ improving hardness and 2$^{nd}$ reducing hardness, cohesiveness and breaking strength. Muguerza *et al.,* (2003) described the addition of soy protein of soy oil did not modify the percentage of water or protein and the pH in fermented sausages with the addition of pre emulsified soy oil cholesterol hardly decreased and oxidation was not modified. Saturated and monosaturated fatty acids decreased and polyun-saturated increased due the significant increase in linoleic and α linolenic acids.

In frankfurters and fish frankfurter analogs, incorporated soy protein hydrolysatessrs reduce a bacterial count and extracted their shelf life stored at 25°C without influencing the flavor and texture properties of the products (Vallejo-Cardossba, *et al.,* 1987). Soy flour produced some beany flavor and soy protein concentrates and isolate provide some undesirable palatability in soy added meat products (Rakosky, 1970; Smith, *et al.,* 1973).

Wilson and Sebranek, Ho, (1997) described tofu powder added to lean meat had lower moisture content, but their overall acceptability and texture was better than control. Soy isolate is a promising source of soluble protein (Sofos and Allen, 1977, Hand *et al.*, 1987). In cooked sausage used soy protein replacement of fat were successfully improved binding properties and had no detrimental effect of sensory characteristics (Serdarroglu and Ozumer, 2003). Arun (2008) described addition of soy protein did not make significant effect on shelf life of goat meat nuggets in frozen storage, while pH, moisture, fat percentage, protein content and water holding capacity were significantly ($p<0.05$) lower in nuggets with 15% soy protein. Also Arun (2008) reported lower force required compressing or shearing the goat meat as hardness, springiness, gumminess and chewiness decreased in soy paste incorporated nuggets.

Potato starches have been used a long time in meat processing during the preparation of sausage and other meat products (Ruban, *et al.*, 2008). The sausages prepared with potato flour had a darker color, the lowest folding score and softer texture (Muthia 2010). Addition of starch to increase the acceptability and quality of meat products (Hughes, *et al.*, 1997; Ahamed, *et al.*, 2007; Nisar, *et al.*, 2009). Murphy (2000) introduced in comminuted meat products potato starches to increase cooking yield and improved texture and extended shelf life. (Claus and Hunt 1991) reported that modified starches are also used to maintain juiciness and tenderness of low fat meat products. The effect of hydrolyzed potato protein (HPP) on meat emulsion, increased fat proportion, lightness, Hue angle and decreased redness, yellowness, chroma, hardness and fracture (Nieto, *et al.*, 2009). Nieto (2009) suggested HPP had antioxidant and emulsifying properties in meat emulsion manufacturing.

The addition of sago flour resulted higher folding score, greater elasticity and increased acceptability of sausage due to higher scores for texture and juiciness (Muthia, 2012). The addition of poly dextrose and out bran was significant decreased in cooking loss of the sausages with the compare control sample (Akesown, 2013). The addition of barley fiber which has the highest content of soluble □- glucan (22.3%), lead to the same high process and frying losses as the addition of rye bran and lowest firmness in sausages, however barley fiber is not good to ingredient in sausage in high content of □- glucum and a large soluble fraction (Petersson, 2014). The rye bran was added in frank further type sausage and meatballs the resulted in sausages meat protein network governs the texture and water holding properties whiles the meat ball had a more particulate structure, with high frying losses and harder texture. Whereas the addition of untreated rye bran to sausages was harmful, causing a remarkable increase in frying loss (Peterson, 2014). Enzymatic treatment of the rye brans didn't improve the WHC or the texture of the sausage to the rye bran only been soaked in water (Peterson, 2014).

The addition of 1% egg white powder improved quality of enrobed buffalo meat cutlets while a 3% level effect on the sensory attributes and lower shrinkage (Ahamed, 2007). Egg white powder combined with flours in duck sausage increased protein content, cooking yield, folding test, WHC, lightness, moisture and fat retention, However decreased significantly the ash and carbohydrate contents of duck sausages (Muthia, 2012). Duck sausages with wheat flour higher protein content ad lightness value and a harder texture, while sausages with potato flour had darker color, the lowest folding scores and softer texture (Muthia, 2010).

Wheat proteins are a high additive due to their ability to form of viscoelastic mass of gluten through the interaction with water (Pritchar and Borck, 1994). Gluten produced from wheat flour can use to binder of sausage products (Jansin-hydrolyzed, *et al.,* 1994). Chymotrypsin-hydrolyzed wheat gluten resulted improve thermal gelation and emulsifying properties of myofibrillar protein isolate and lower microbial tansglutaminase activity (Xiong *et al.,* 2008). Li, Carpenter and Cheney (1998) reported the addition of 3% and 6% of wheat proteins to smoked sausage of poultry meat the product increased hardness and decreased springiness.

Dietary fiber is defined as the remnant of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in human small intestine (Prosky, 1999). Addition of 17% and 29% of peach dietary fiber suspensions to frankfurter increased viscosity and decreased pH without influencing cooking loss, protein content, collagen contents, and sensory evaluation of sausages (Gringelmo-Miguel *et al.,* 1999). Garcia *et al.,* (2002) reported the addition of high level of cereal and fruit fibers increased hardness and cohesiveness and decreased sensory and textural properties in low fat and dry fermented sausage. The addition of 1% and 2% of orange fiber to Spain dry fermented sausage increased amount of micrococcus and deceased the residual of nitrite during fermentation (Fernandez *et al.,* 2008).

Allicin is the main ingredient of garlic that has antimicrobial activity against both gram-positive and negative bacteria. The addition of 1% and 3% garlic juice decreased peroxide value TBAR, residual nitrite and microbiological counts in emulsion sausage during cold storage (Park and Kim, 2009). Catechins is a predominant group of polyphenols present in green tea leaves composed of four compounds epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallet (Zhong *et al.*, 2009). Green tea providing antibacterial, anticarcinogenic and antivirability (Yang *et al.*, 2000). The addition of green tea catchins at the level of 300ppm reduced the TBARS values of beef, duck, ostrich, pork and chicken during 10 days refrigerated storage and also providing two to four time anti oxidative abilities then α-tocopherol depend on different animal spices meats (Tang *et al.*, 2001). Choi *et al.*, (2003) reported the addition of green tea powder in pork sausage resulted in lower TBARS and decreased voltaic basic nitrogen contents compared sample prepared with nitrate alone.

Clove oil at 0.5% and 1% level inhabited the growth of L.monocytogenes in minced muttons. At the level of 1% number of L. Monocytogenes decreased by 1-3 log cfu/g in mutton (Menon and Carg, 2001). Clove was able to prevent discoloration of raw pork during the storage at the room temperature lipid oxidation among spices and herb extract (Shan *et al.*, 2009).

Compounds from herbs and spices contain many phytochemicals, which are a potential source of natural antioxidants including phenolic diterpenes, flavonoids, phenolic acid and tannins (Dawidowicz *et al.*, 2006). Herb and spice compounds have antioxidant, anti-inflammatory and anticancer activity. Among the all spices, cloves have strongest antioxidant capacity followed by rose petals, cinnamon, nutmeg and other spices (Al. Jalay *et al.*, 1987). Additions of spices have antimicrobial ability mainly due to phenolic compounds. Rosemary extract contains high level of phenolic compounds and its great antioxidant activity. Addition of rosemary extract to pork sausage at the level 2500 ppm equal to or effective than BHA/BHT inderlying TBARS value in raw and pre cooked sausage during refrigerated and frozen storage, also addition of rosemary extract improved the color and freshness of pork sausage (Sebrank *et al.*, 2004).

In increasing of phosphate level caused alleviatable effects in increased hardness emulsion sausage made by pre- rigor meat, but opposite effects made with frozen meat (Peng, 2009). The addition of natural phenolic- rich plant extract in the production of bologna type sausages found to protect against some types of oxidation in the sausages but not against thiol modifications (Jongberg, 2013). Lipid oxidation effectively inhibited by the addition of green tea extract or rosemary extract, and correlated positively with the protective effects against protein carbohydrate formation (Jongberg, 2013). Also reported appraise of protein thiols and protein polymerization showed that the green tea extract increase thiol loss and the plant extracts were unable to prevent the protein cross link formation of the myosin heavy chain. Tannic acid 0.02%, 0.04% and Ehanolic Kiam Wood Extract (EKWE) 0.08% effected in postponing the lipid oxidation by lower TBARS formation as well as lower development of fishy odor, while addition of EKWE at the level of 0.04% was not efficient in preventing the lipid oxidation in the fish emulsion sausages (Maqsood, 2012). The addition of tannic acid at the level of 0.04% maintained the textural properties to a high extent after 20 days storage at 4°C (Maqsood. 2012).

# CHAPTER-3

# Materials & Methods

# CHAPTER-3

# MATERIALS AND METHODS

Experimental work was carried out to study the effect of incorporation of whey protein products on the quality of the buffalo meat emulsion sausage. The quality of sausages developed were evaluated on the basis of physico-chemical characteristics (pH, moisture content, protein content, fat content, water holding capacity (WHC), ash content, extract relies volume (ERV) and TBA (Thiobarbituric acid) number), microbiological characteristics (total plate count, yeast and mold count, coliform count and salmonella shigella ) sensory characteristics (colour, flavor, texture, taste, mouth coaching, palatability and juiciness). This chapter presents the details of materials and methods used in present investigation.

### 3-1 Buffalo meat

Meat samples collected from the local meat shop in the study were from buffaloes slaughtered according to the traditional halal method at the slaughter house of the municipal corporation, Aligarh. Meat samples from a round portion (biceps fermoris muscle) of 2.5, 3 and 3.5 years aged female carcasses of good finish were obtained from the meat shop within 4 hr. of slaughter. The meat chunks were packed in combination film packaging and brought to the laboratory within 20 min. Buffalo fat was also packed in combination film packaging and brought to the laboratory. The connective tissue portions of the samples were removed. Other non-meat ingredients like spices, salt, condiments, casings and LDPE film were procured from the local market. Whey protein concentrate provided by Mahaan proteins Ltd, New Delhi, India, and whey protein isolate and whey protein powder provided from market. The meat and fat were kept inside ultra low temperature cabinet (Yarco, India) at $2^{o}$ C for 20 hours.

### 3.2 Equipment, apparatus and instrument used

A number of equipment and instrument were required to conduct the present study. These include bowl chopper, sausage filler, stuffing for production of sausage, digital pH meter for pH measurement (Thermo Orion USA), electronic balance (Anamed, India), high speed tissue homogenizer (Yarco, India), heat sealing machine (Quick Seal, Sevana, India), hot air oven (Tanco India), Soxhlet apparatus for fat estimation (Borosil, Germany), Digital spectrophotometer (Digital spectrophotometer

Model 310E, India), BOD cum humidity incubator (Yarco, India), protein analysis (DK6 heating digester,VELP Scientifica, Europe), mincer (Sirman TC22, Glasgow Scotland), refrigerator (Remi quick freezer, New Delhi), Laminar flow for microbial preparation (Yorco, India), and Autoclave (Pooja scientific instrument, New Delhi; PSI-101).

### 3.3 Meat sample preparation

Twenty six Kg meats were taken for preparation of emulsion sausage. Four different type samples of emulsion sausages namely controlled, emulsion sausage incorporated with whey protein concentrate, isolate and whey protein powder were prepared. The sample size was kept 2.0 Kg lean meat each (excluding other ingredient like fat, whey protein, salt, spices and condiment). Different levels of whey protein products (Whey protein concentrate, isolate and whey protein powder) 1, 2, 3, and 4% was taken for the study of emulsion sausage. Controlled sample and three treatments each contain four levels (1, 2, 3, and 4% whey protein products[1]) were prepared.

### 3.4 Preparation of spices and condiments

The formulation of spices mix in powder form was kept in the following ratio:

| Spices ingredients | g in the mix |
|---|---|
| Black paper | 60 |
| Red chili pepper | 50 |
| Cumin seed | 100 |
| Cinnamon | 30 |
| Javitri (Mace) | 30 |
| Cardamom | 15 |
| Cloves | 15 |

Garlic (250 g), ginger (100 g) and onion (250 g) paste was prepared by first peeling of the external covering and weighed in equal quantity. They were cut into small pieces and ground in a laboratory grinder (Braun co, India) into a fine paste.

[1] Whey protein concentrate, isolate and powder

### 3.5 Preparation of emulsion sausage

Emulsion sausages were prepared from a comminuted mixture of meat; fat, salt, spices, condiments and incorporated with different levels (1, 2, 3 and 4%) of whey protein products. The composition of emulsion sausages was kept as below:

**Formulation of emulsion sausage incorporated with whey protein products**

| | |
|---|---|
| Lean meat | 2.0kg |
| Fat[2] | 400g |
| Whey proteins[3] | 600g |
| Spices | 32g |
| Condiments | 50gm |
| Salt | 45g |
| Chili powder | 15g |
| Ice | 100g |
| Mono sodium glutamate | 3g |
| Sodium ascorbate | 500 ppm |
| Sodium nitrate | 150ppm |
| Casing | 25 mm dia. |

The buffalo meat was ground on a grinder (PRS Technologies, India) at (11°C temperature, through a 0.95cm plate). The ground meat was transferred to bowl chopper (PRS Technologies, India) for further Comminution. It was chopped at slow speed (17 rpm) for two minutes, and then ice cubes (50 g) were added and further comminuted for two minutes. As the mix absorbed the moisture received from molten ice, the other ingredients like fat, salt, spices, condiment and whey protein were added and chopping was further continued for five minutes and the remaining ice addition brought temperature in range of 14-16° C during chopping. Entire mix was filled in the stuffing machine (PRS Technologies, India) and collagen casing (25mm dia) was used for filling sausage. The finished sausage was cooked in sausage cooker (Yarco, India, operated by steam) for 20min at 110°C temperature. Cooked sausages were exposed to chilled water or chill water was spread over cooked sausage. This operation led to the cracking of casing and finally the sausages were packed in Low

[2] Formulation is based on the 20% fat

[3] Formulation is based on the 1, 2, 3, and 4% of whey proteins products (whey protein concentrate, isolate and whey whey powder)

sity polyethylene (LDFE) packaging. The finished sausages were stored at 0°C in ıltra low temperature cabinet for future study. Fig 3.1 shows the emulsion sausage ırporated with whey protein.

ıle 3.1: Work plan and parameters of study

| oduct | Ingredients | Level of fat | Levels of whey proteins | Casing and Packaging materials | Storage |
|---|---|---|---|---|---|
| alo : lsion ıge | Buffalo meat, fat, Condiments, salt, spices, ice, chili powder, mono sodium glutamate, Sodium ascorbate, Sodium nitrate Whey proteins (concentrate, isolate, whey protein powder) | 20% of animal fat | 1, 2, 3 and 4% of whey protein concentrate, isolate and powder in each treatment. | Collagen casing and LDPE film packaging | 0°C in an ultra low temperature cabinet |

ıle 3.2: Studies on properties of emulsion sausage

| ıage pe | Physico-chemical characteristics | Microbial characteristics | Sensory characteristics | Remark | Instrumental colour measurement |
|---|---|---|---|---|---|
| alo : lsion ıge | Moisture content<br>pH<br>Ash content<br>Protein content<br>Fat content<br>TBA number<br>Water holding capacity<br>Extract release volume | Total plate count (TPC)<br>Yeast and mold count<br>Coliform count<br>Salmonella shigella | Color<br>Aroma<br>Texture<br>Taste<br>Mouth coating<br>Juiciness<br>Palatability | Like extremely<br>Like very much<br>Like moderately<br>Like slightly<br>Neither like nor dislike<br>Dislike slightly<br>Dislike moderately<br>Dislike very much<br>Dislike extremely | Colour measurement by Hunter Lab |

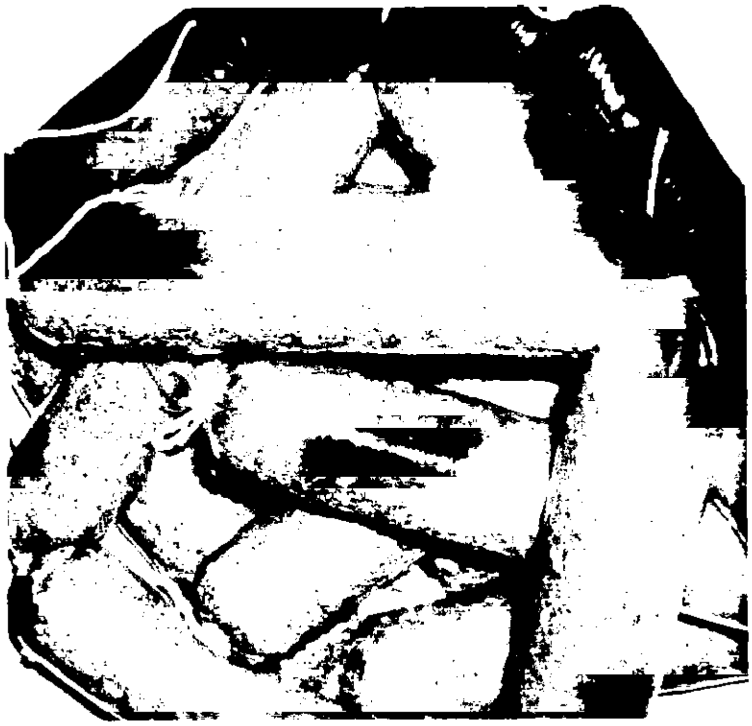


**Fig 3.1**: Buffalo meat emulsion sausage incorporated with whey protein

**Fig 3.2:** Meat and nonmeat ingredient used in emulsion formation in bowl chopper

### 3.6 Evaluation of properties

Samples of raw buffalo meat were analyzed for moisture, protein, fat, ash contents and pH (AOAC, 1990). Initial total plate count and yeast and mold counts of these samples were also assessed (APHA, 1992). The quality characteristics of emulsion sausages were evaluated at constant intervals of days during refrigerated storage (at 0° C). Emulsion sausages were shelf stable products and therefore could be safely kept under refrigerated condition for several weeks.

#### 3.6.1 Evaluation of physico- chemical properties

##### 3.6.1.1 Estimation of moisture

Moisture content of sausages samples were evaluated as given in Food Industry Manual (Ranken et al., 1993). 10 g of sausages was weighed into flat-bottom, dried petri dishes. These dishes and its content were placed in hot air oven (Yorco, India) thermo statistically controlled at 150 (5° C for 4 hours and taken out and further heated until successive weighing showed no further loss. At the end, the dishes were removed from the oven and placed in a desiccator allowed to cool and weighed. Following formula was used for the estimation of moisture content of sausage samples.

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$

### 3.6.1.2 Estimation of ash

The sample was weighed and ignited at the temperature 550° C for 6 hours in the crucible furnace (Yorco, India). It was then taken out and allowed to cool for a moment and placed in desiccators until cooled and finally weighed to a constant weighed.

$$\text{Ash content (\%)} = \frac{\text{Final weight of ash}}{\text{Initial weight of sample}} \times 100$$

### 3.6.1.3 Protein estimation

Protein was analytically estimated by determining the amount of total nitrogen in the sample.

Amount of protein in the sample = total nitrogen x 6.25

**Reagents**

1) Sulphuric acid, 98% pure
2) 0.1 N Hydrochloric acid
3) 2% Boric acid solution
4) 40% Sodium hydroxide.
5) Catalyst mixture (2.5 g Selenium oxide + 110 g Potassium sulphate and 20 g copper sulphate)
6) Mixed indicator (Bromo cresol green 0.1 % + Methyl red 0.1%

2 g of finely minced emulsion sausage was transferred in to digestion tubes and 2 g of catalyst mixture was also added. Then 25 ml concentrated sulphuric acid was poured into the mixture and kept for digesting in DK6 heating digester (VELP Scientifica, Europe) for 3 hours. At the end, the mixture becomes colorless.

After cooling the tubes were transferred to Kjeldhal apparatus. The ammonia liberated from the reaction mixture was absorbed in 20 ml of 2% boric acid solution. Distillation was continued for five minutes. This solution was titrated against 0.1N HCl using mixed indicator. The blank was running in the second test of experiment and the titration was done in a similar way.

**Calculation**

$$\text{N (\%)} = \frac{(\text{Sample} - \text{Blank}) \times \text{N of HCl} \times 14 \times 100}{\text{weight of sample} \times 1000}$$

Protein content (%) = 6.25 x N

#### 3.6.1.4 Fat estimation

(a) Apparatus: Soxhlet apparatus

(b) Solvent: Petroleum ether as extraction medium

**Method**

10 g of emulsified sausage was taken and kept the thimble. The thimble was placed in the extracting tube and this tube was connected with the weighted flask and also the condenser.

The heat vaporized the volatile solvent, which passed up the side arm and was condensed in the condenser. The condensed solvent fell drop by drop onto the thimble. When sufficient amount of solvent had thus been transferred to the extracting tube to fill the siphon arm, it siphoned back over into the weighed flask. This process was continued for 90 min until the extraction was completed. Then the bottom flask was removed, the volatile solvent was evaporated and fat extracted was obtained as a residue. The following formula was used to express the fat content of the sample.

$$\text{Fat content (\%)} = \frac{\text{weight of residue left after evaporation of solvent}}{\text{weight of sample}} \times 100$$

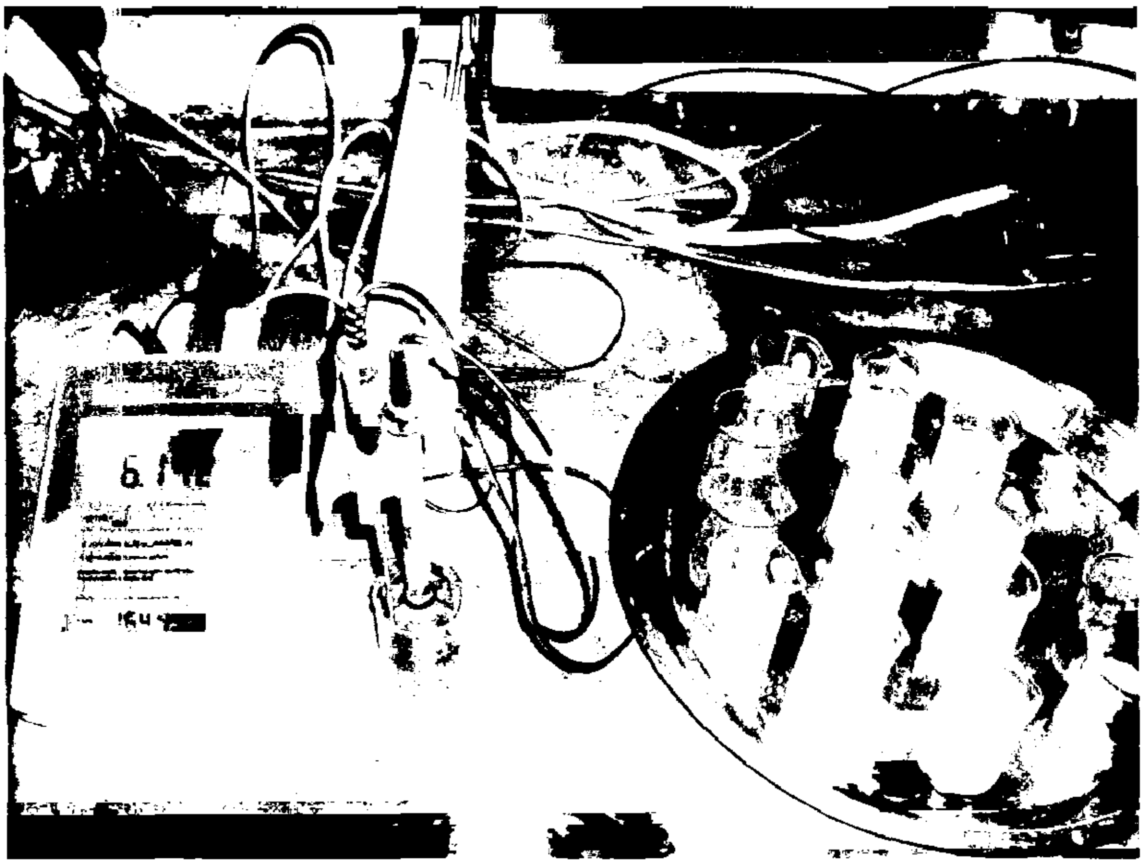


Fig 3.3: pH measurement

### 3.6.1.5 pH Measurement

The pH of the finally minced samples was determined after homogenizing 10g of the sample with 100 ml distilled water using a laboratory grinder (Yarco, India). The pH of the suspension was recorded using reference and glass electrode portable type Digital pH meter model PH1500 (Eutech, Singapore).

### 3.6.1.6 Estimation of thiobarbituric acid (TBA) number

**Reagents**

1) Tricarboxylic acid reagent (TCA) 20%.
2) Tricarboxylic acid reagent (TCA) 10%.
3) Acetic acid, 90%.
4) TBA reagent.

**Method**

Thiobarbituric acid TBA reagent was prepared by dissolving 0.2883 g of Thiobarbituric acid in sufficient quantity of 90% acetic acid and by slight warming, the volume being made up 100 ml with 90% acetic acid.

20 g of meat sample of sausage was blended in a blender with 50 ml of cold 20% tricarboxylic acid (TCA) for 2 min. The blended contents were rinsed with 50 ml of distilled water, mixed together and filtered through filter paper and the interstate was collected in a 100 ml capacity-measuring cylinder. The filtrate, termed the TCA extract was used in the estimation of thiobarbituric acid (TBA) number.

TBA number was measured by the method described by Strange et al., (1977). Five ml of TCA extract was mixed with 5ml of TBA reagent in a test tube. The test tube was kept in a water bath at 100° C for 30 min along with another test tube containing a blank of 10% TCA and 5 ml of TBA reagent. After cooling the tubes in running water about 10 min, the absorbance was measured at 530 nm in a spectrophotometer (Digital spectrophotometer Model 310E, India) and reported as TBA number.

### 3.6.1.7 Determination of extract release volume (ERV)

ERV of meat samples was evaluated as given in manual for analysis of meat and meat products (Food safety and standard authority, 2012).

Homogenized 20 gm sausage sample with 100 ml of distilled water for 2 min. Pour the homogenate direct in to the panel lined with whatman filter paper No.1,.

Allowed the homogenate and collected extract in 100 ml graduated cylinder for 15 min and recorded extract release volume.

**Interpretation**

| ERV (ml) | Sausage quality |
|---|---|
| >25 ml | good quality |
| >20 ml | incipient spoilage |
| <20 ml | spoilage sausage |

**3.6.1.8 Water holding capacity (WHC)**

Water holding capacity was determined according Lianji and Chan (1991). 10 gm of sausage sample placed in glass jars and heated at 90°C for 10 min in a water bath. After heating sample was carefully removed from glass jars and cooled to room temperature, wrapped in cotton cheesecloth, and placed in 10 ml polycarbonate centrifuge tubes, then centrifuged 9000 rpm in 4°C for 10 min. After centrifuging sample were re weight and WHC was calculated below formula.

$$\%WHC= 1\text{-}T/M\times100= 1\text{-}B\text{-}A/M\times100$$

T= total fluid loss during heating and centrifugation

M= total water content in the sample

B= weigh of the sample before heating

A= weight of sample after heating and centrifugation

**3.6.2 Evaluation of microbiological characteristics**

All the samples were evaluated for the direct plate count using serial dilution spread plate technique with nutrient agar medium for total plate count and potato dextrose agar for yeast and mold count, MacConkey agar for coliform count and S.S. agar for *salmonella shigella* count (APHA, 1992). The microbiological characteristics of sausage samples were evaluated in fresh conditions and during refrigerated storage ($0^{o}$ C) after constant intervals. For the determination of the total plate count, yeast and mold count, coliform count and *salmonella shigella* count, the samples were taken with sterile knife, committed to fine particles in a tissue Homogenizer (Yarco, India) and then transferred to a test tube containing 9 ml of normal saline solutions. The samples were homogenized in the cyclomixer (mode CM-101, India). Serial dilutions were made by transferring 1 ml of the extract from each dilution and finally the samples were inoculated in the petridishes containing the solid medium. The colonies were counted after 24-48 hr incubation in BOD incubator (York Scientific, India).

$$\text{TPC (cfu/g)} = \frac{\text{Number of colonies}}{\text{Amount used for inoculation} \times \text{dilution factor}}$$

### 3.6.3 Evaluation of sensory characteristics

Sensory attributes such as color, flavor, texture, taste, mouth coating, juiciness and palatability of the emulsion sausage samples were evaluated as recommended by Ranganna (1994) by Hedonic rating test. A trained panel consisting of 10 expert judges was selected to evaluate the samples through properly planned experiments. The panelists were selected from the staff and students of the Department of Post Harvest Engg. & Technology, Faculty of Agricultural Sciences, Aligarh Muslim University (AMU), Aligarh.

Samples were served to the panelists and they were asked to rate the acceptability of the product through the organs of sense. Different attributes viz. colour, flavor, texture, taste, mouth feel and juiciness of the emulsion sausages were rated on the basis of 9 points of the hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like). A test Performa was also made and supplied to the panelists at the time of evaluation. It is given here, 9= like extremely, 8= like very much, 7= like moderately, 6= like slightly, 5= neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much, 1= Dislike extremely.

### 3.6.4 Instrumental measurement of colour by Hunter Lab

The sample of semi dry fermented sausage made flat by pressing hard. The Hunter Lab instrument was kept over the samples. The value (L), hue (a) and chroma (b) were measured in numerical value by digital system of the device.

### 3.7 Statistical analysis

Data obtained from experimental observation (n=5), were subjected to analysis of variance (Two ways ANOVA) variance (Cochron and Cox, 1992). Linear regression was also determined to study the storage behavior of physic-chemical, microbial and sensorial properties of emulsion sausage incorporated with different levels 1, 2, 3 and 4 % of whey protein products.

### 3.7.1 Analysis of Variance

To test the significance of the effect of treatment and storage period on quality parameters, analysis of variance (ANOVA) of the collected data for different properties was carried out (Dospekhov, 1984). The least significant differences (LSD) or critical differences (CD) of mean values were calculated using formula

$$\text{LSD or CD} = \left[\sqrt{\frac{2 \times S^2}{n}}\right] \times t$$

Where,

S= error of total sum of deviation squares

n= No. Of replications

t= value at 0.05 obtained at the degree of freedom

**Fig 3.4:** Sensory evaluation carried out by panel of M.Tech students

**Fig 3.5:** Preparation of the emulsion sausage

**Fig 3.6:** Sensory evaluations

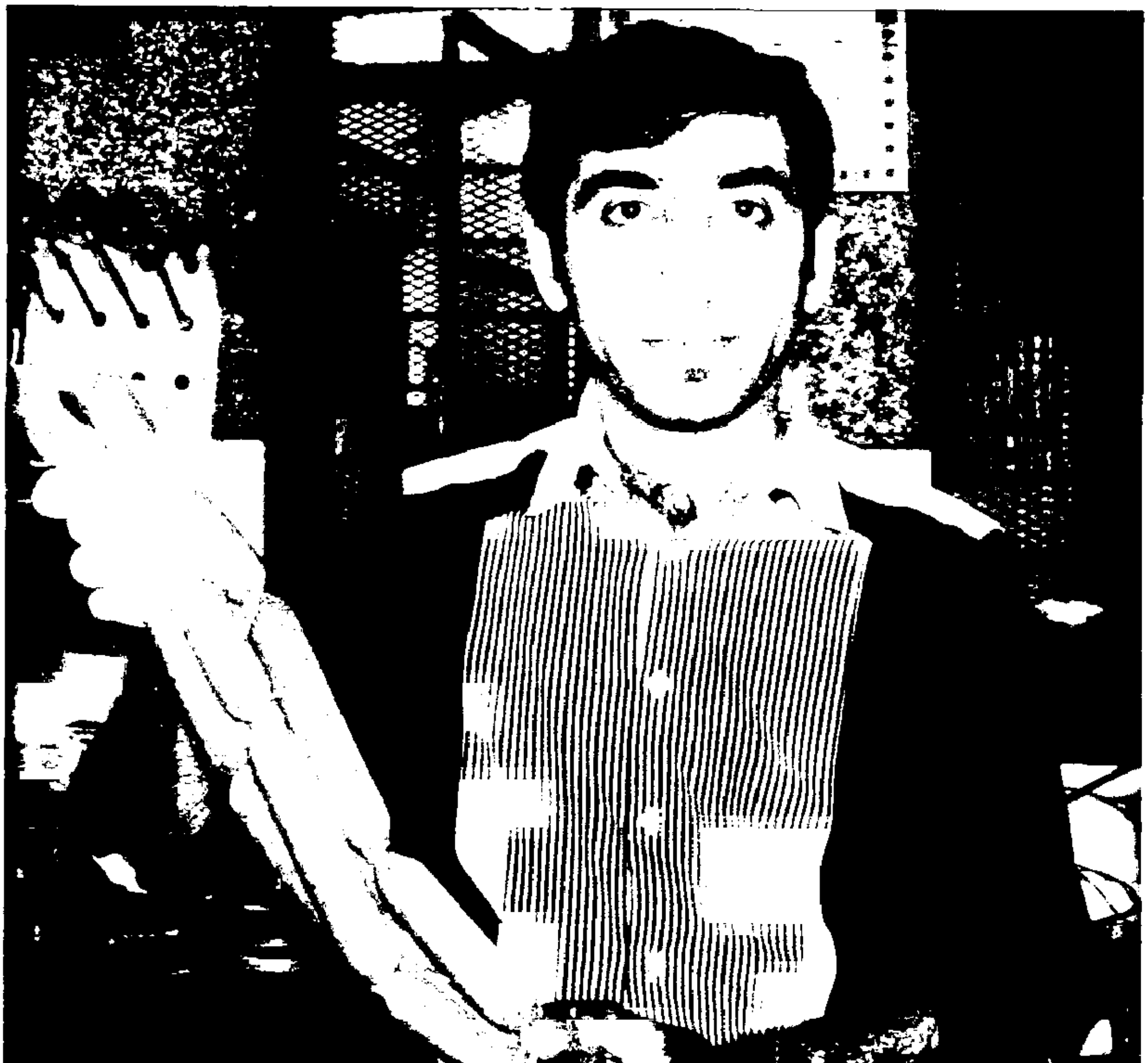


Fig 3.7: Emulsion sausage

# CHAPTER-4

# Results and Discussion

# CHAPTER-4

# RESULTS AND DISCUSSION

The present study was carried out to study the effect of incorporation of whey protein products on quality and shelf life of buffalo meat emulsion sausage during refrigerated storage (0°C). Three treatment of whey protein product namely whey protein concentrate, whey protein isolate and whey protein powder were selected with each four level of 1, 2, 3 and 4%. Thirteen samples (three x four+ one control) of emulsion sausages were prepared.

The quality of sausage developed was established on the basis of physico-chemical characteristics (moisture content, pH value, ash content, protein content, Thiobarbituric acid (TBA) number, fat content, extract release volume (ERV) and water holding capacity (WHC), microbiological characteristic (total plate count, yeast and mold count, coliform count and *Salmonella shigella* count), sensory characteristics (colour, aroma, texture, taste, mouth coating, juiciness, palatability and overall acceptability) and instrumental colour. For evaluation of shelf life of emulsion sausage during refrigerated storage (0°C), these quality measuring characteristics were estimated after every 5$^{th}$ day till the end of shelf life/ beginning of spoilage condition of sample. The criteria of shelf life and finally spoilage condition were defined on the basis of microbiological characteristics, thiobarbituric acid number and sensory characteristics. The results and discussions related buffalo meat emulsion sausage incorporated with different levels of whey protein products have been presented in this chapter separately under appropriate sub section respectively.

### 4.1 Analysis of raw buffalo meat

The proximate composition of raw buffalo meat obtained after chemical analysis has been presented in Table 4.1. The raw buffalo meat has 74.2% moisture content, 6.9% fat, 18.2% protein and 1.1% ash content. Carbohydrate was absent in lean meat. The pH value of raw buffalo meat was 6.14 and log TPC/g value was 3.1 while yeast and mold, coliform bacteria and *Salmonella shingella* were absent in the lean meat.

**Table 4.1:** Analysis of buffalo meat as raw material for buffalo meat emulsion sausages.

| [illegible] | [illegible] |
|---|---|
| Moisture content | 74.2±0.619 |
| Ash content | 1.1±0.093 |
| Protein content | 18.2±0.030 |
| Fat content | 6.9±0.187 |
| Carbohydrate | - |
| pH | 6.14±0.115 |
| Total plate count (log TPC/g) | 3.1±0.116 |
| Yeast and mold count | ND** |
| Coliform count | ND |
| *Salmonella shigella* count | ND |

* Values are means of five replicate± SD

**ND: Not detected

## 4.2 Effect of incorporation of whey protein products on quality of buffalo meat emulsion sausage

### 4.2.1 Development of emulsion sausages

The emulsion sausage were developed from buffalo meat using different levels (1, 2, 3 and 4%) of whey protein products namely whey protein concentrate, isolate and wheý protein powder. The effect of different levels of whey protein products including control on physicochemical characteristics namely moisture content, pH value, ash content, protein content, fat content, TBA number, ERV and WHC, microbiological characteristic (total plate count, yeast and mold count, coliform count and *Salmonella shigella* count), sensory characteristics (colour, aroma, texture, taste, mouth coating, juiciness, palatability and overall acceptability) instrumental colour of the products were examined after preparation.

#### 4.2.1.1 Physico chemical characteristics of fresh emulsion sausages incorporated with whey protein products

**(i) Moisture content**

Moisture content of fresh emulsion sausage is an important characteristic, which relates to the quality and shelf life. It also influences storage stability and texture of foods. Furthermore high moisture foods are more prone to microbial spoilage, but they have a softer texture.

**a) Emulsion sausage incorporated with whey protein concentrate**

Tables 4.2 present the results of moisture content of buffalo meat emulsion sausage incorporated with different levels (1, 2, 3 and 4%) of whey protein concentrate (WPC). The moisture content of control sample was 63.40% and the sample of emulsion sausage treated with whey protein concentrate with different levels (1, 2, 3 and 4%) had moisture contents 64.54, 64.82, 64.70 and 64.40% respectively. It was shown that the addition of whey protein concentrate slightly increased the moisture content of samples as compared to control sample. Different levels of WPC significantly ($p<0.05$) effect the moisture content of the emulsion sausage samples. Meltem and Eylem (2004) also found similar results; this results indicated that incorporation of dairy ingredients significantly increased ($p<0.05$) moisture content of sausage samples and these results also are in agreement with Hung & Zayas, (1992).

**b) Emulsion sausage incorporated with whey protein isolate**

The moisture content of fresh emulsion sausage samples with 1, 2, 3 and 4% have been presented in Table 4.3. The moisture content of control sample was 63.4% and fresh sample of emulsion sausages with incorporation of whey protein isolate (WPI) with different levels (1, 2, 3 and 4%) were found to be 64.21, 64.10, 64.04 and 64.08% respectively. Addition of whey protein isolates slightly ($p<0.05$) increased the moisture content of emulsion sausages incorporated with whey protein isolate as compared to control sample. Increasing the levels (1, 2, 3, and 4%) of whey protein isolate significantly ($p<0.05$) decreased the moisture content of emulsion sausage samples.

**c) Emulsion sausage incorporated with whey protein powder**

The results of moisture content of buffalo meat emulsion sausage incorporated with whey protein powder (WPP) with different levels (1, 2, 3 and 4%) have been presented in Table 4.4. In fresh condition the moisture content of control sample was 63.40% and that of emulsion sausage incorporated with of whey protein powder with levels of 1, 2, 3 and 4% was found 65.70, 64.71, 66.12 and 64.96% respectively. It showed that the addition of whey protein powder increased the moisture content of samples as compared to control sample. Similar results were found by Andic *et al.,* (2004). He advocated that whey powder and skim milk powder (SMP) significantly increased ($p<0.01$) moisture retention values of meat patties. He also also explained that patties formulated with WP had higher moisture retention than those formulated with SMP and control samples. Similarly Ulu (2004) found that result by addition of 0.2% WP increased moisture content of meatball. Different levels of WPP significantly ($p<0.05$) effect the moisture content of the emulsion sausage samples.

**(ii) pH value**

The pH is one of the important characteristics of meat products. The pH value has a significant impact on colour, shelf life, taste, microbiological stability, yield and texture of meat and meat products. The pH value referred to acidity of meat and it is indicating the extent of post mortem glycolysis.

**a) Emulsion sausage incorporated with whey protein concentrate**

The results of incorporation of whey protein concentrate with different levels (1, 2, 3 and 4%) have been presented in Table 4.2. It was found that, the pH value of the emulsion sausage was a litter higher than fresh meat. The pH value of control sample was 6.39. Incorporation of whey protein concentrate brought a slight change in pH of emulsion sausage. Addition of whey protein concentrate to emulsion sausages made a slight decrease of pH of sausage samples as compared to the control sample. Although, these results differ from Sammel and Claus (2003), this study indicated that incorporation of WPCs increased pH value of cooked ground turkey breast significantly ($p<0.05$) as compared to the control sample. Szerman *et al.,* (2008) reported that the pH increased linearly with WPC until the maximum concentration studied (3.5%). With increasing the levels of whey protein concentrate, pH values of emulsion sausage samples were found to increase significantly ($p<0.05$).

**b) Emulsion sausage incorporated with whey protein isolate**

The results of pH values of emulsion sausage incorporated with different levels of whey protein isolate have been presented in Table 4.3. The pH value of control sample was 6.39. The pH value of emulsion sausage incorporated with whey protein isolate at the levels of 1, 2, 3, 4% were found to be 6.36, 6.31, 6.29 and 6.28 respectively. Incorporation of whey protein isolate brought a slight change in pH value of emulsion sausage. Different levels of WPI significantly ($p<0.05$) affected the pH values of the emulsion sausage samples.

**c) Emulsion sausage incorporated with whey protein powder**

The results of evaluation of pH of buffalo meat emulsion sausage incorporated with different level whey protein powder (1, 2, 3 and 4%) have been presented in Table 4.4. The pH value of control sample was 6.39 and that of emulsion sausage incorporated with whey protein powder at the levels of 1, 2, 3 and 4% was found 6.31, 6.31, 6.35 and 6.36 respectively. Incorporation of whey protein powder brought a slight change in pH of emulsion sausage. This also accords with our earlier observations (Badpa and Ahmad, 2014b) which showed that addition of whey protein concentrate and isolate brought a slight change the pH value of samples compared to the control sample.

**(iii) Ash content**

The ash content of emulsion sausage showed the quantity of mineral matter in the samples.

**a) Emulsion sausage incorporated with whey protein concentrate**

The results of evaluation of ash content of buffalo meat emulsion sausage (ES) incorporated with whey protein concentrate (WPC) at different level (1, 2, 3 and 4%) have been presented in Tables 4.2. In fresh condition the ash contents of emulsion sausages of control sample was 2.36% and samples incorporated with whey protein concentrate at levels of 1, 2, 3 and 4% had 2.11, 2.13, 2.15 and 2.17% respectively. There was a significant ($p<0.05$) difference in ash contents of control sample and emulsion sausages incorporated with whey protein concentrate. Incorporation of WPC significantly ($p<0.05$) decreased the ash content of emulsion sausages. However, increasing levels of whey protein concentrate increased the ash content of ES. This was may due to that mineral of meat polymerized with the mineral of WPC.

**b) Emulsion sausage incorporated with whey protein isolate**

The results of ash content of emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%) have been presented in Table 4.3. The ash content of control sample was 2.36. The ash contents of emulsion sausage treated with whey protein isolate with different levels (1, 2, 3 and 4%) were found 2.29, 2.25, 2.20 and 2.21% respectively. It showed that the whey protein isolate slightly decreased the ash contents of emulsion sausage. Different levels of WPI significantly ($p<0.05$) affected the emulsion sausage samples.

**c) Emulsion sausage incorporated with whey protein powder**

The results of evaluation of ash content of control sample and buffalo meat emulsion sausage (ES) incorporated with whey protein powder with different level (1, 2, 3 and 4%) have been presented in Table 4.4. In fresh condition the ash contents of emulsion sausages of control sample was 2.36% and samples treated with whey protein powder at levels of 1, 2, 3 and 4% had values of ash contents as 2.36, 2.18, 2.54 and 2.34% respectively. Different levels of WPP significantly ($p<0.05$) affected the emulsion sausage samples, but incorporation of 1% WPP didn't bring change in ES as compare to control samples.

**iv) Protein content**

Protein content of emulsion sausage is indicator of nutritional value as meat protein is a good source of essential amino acids. Further, addition of whey protein concentrate increased its nutritional value.

**a) Emulsion sausage incorporated with whey protein concentrate**

The results of protein content of buffalo meat emulsion sausage incorporated with whey protein concentrate with different level (1, 2, 3 and 4%) have been presented in Tables 4.2. The protein content of control sample of emulsion sausage was found to be 22.58% and sample of sausage with incorporation of whey protein concentrate at levels of 1, 2, 3 and 4% were found to be 23.58, 23.55, 24.06 and 24.10% respectively. It showed that the whey protein concentrate significantly ($p<0.05$) increased the protein contents of emulsion sausage. Increasing levels of WPC, increased the protein content of samples. This result may be explained by the fact that the whey protein concentrate has high protein content of order 70.02%.

**b) Emulsion sausage incorporated with whey protein isolate**

The protein contents of emulsion sausages incorporated with different levels (1, 2, 3 and 4%) whey protein isolate were found to have 23.38, 23.84, 24.27 and 24.42% respectively (Table- 4.3). The protein content of control sample was 22.58%. It showed that the whey protein isolate significantly ($p<0.05$) increased the protein contents of emulsion sausage. Fresh sausage samples significantly ($p<0.05$) affected with increasing levels of WPI.

**c) Emulsion sausage incorporated with whey protein powder**

Tables 4.4 presents the results of protein content of buffalo meat emulsion sausage incorporated with different levels (1, 2, 3 and 4%) of whey protein powder. The protein content of control sample was 22.58% and the sample of emulsion sausage treated with whey protein powder with different levels had protein contents 24.90, 24.96, 25.12 and 25.22% respectively. It showed that the whey protein powder significantly ($p<0.05$) increased the protein contents of emulsion sausage. All the levels of WPP significantly ($p<0.05$) affected the protein content of emulsion sausage samples.

**v) Fat content**

Fat content is the important ingredient for making emulsion in sausage, it has high calorific value it provides energy to the body and gives palatability to the product.

**a) Incorporated with whey protein concentrate**

The fat contents of emulsion sausages treated with whey protein concentrate at the levels of 1, 2, 3 and 4% were found to have 12.95, 12.84, 12.73 and 12.69% respectively (Table- 4.2). The fat content of control sample was 13.47%. There is a significant difference in fat contents of control sample and treated sample of emulsion sausages. The result shows a significant ($p<0.05$) for fat content of fresh emulsion sausages.

**b) Emulsion sausage incorporated with whey protein isolate**

The results of fat content of buffalo meat emulsion sausage (ES) treated with whey protein isolate with different level (1, 2, 3 and 4%) have been presented in Tables 4.3. In fresh samples, the fat content of control sample of emulsion sausage was found to be 13.47%; with the whey protein isolate treated samples of sausage had fat content 11.99, 11.73, 11.43 and 11.21% respectively. There were a significant ($p<0.05$) difference between control sample and emulsion sausages incorporated with whey protein isolate. The result shows addition of whey protein isolate a significantly ($p<0.05$) decreased of fat content of emulsion sausage incorporated with whey protein

: with level of 1, 2, 3 and 4% compare with control sample. With increasing the of WPI respectively decreased fat content of emulsion sausage samples.

**ulsion sausage incorporated with whey protein powder**

The results of the fat content of controlled and emulsion sausage have been ted in Table 4.4. The fat content of control sample of ES was 13.47% and the l samples with whey protein powder at the levels of (1, 2, 3 and 4%) were found e 12.23, 12.34, 12.20 and 11.95% respectively. Fat contents of ES incorporated VPP was found to different significantly ($p<0.05$) with control sample. Different (1, 2, 3 and 4%) of whey protein powder incorporation significantly ($p<0.05$) sed the fat contents of emulsion sausage.

**A number**

The sausages contain good quantity of fat and therefore the products is prone d oxidation, which leads to onset of rancidity and finally warm over flavour. arbituric acid number is estimated to know the extent of oxidation of fat. In e food system 2-thiobarbituric acid (TBA test, Melton, 1983) is a measure of ity of fat. The extent of oxidative rancidity is normally expressed in terms of numbers or value (mg per kg. of malonaldehyde of sample). Malonaldehyde is in meat products containing oxidizing polyunsaturated fatty acids and reacts BA to produce a coloured complex with absorption maximum at 530-532 nm. ed pigment obtained is due to the reaction results from condensation of two of TBA with one mole of malonaldehyde (Sinnhuber *et al.*, 1958). The ity of colour was originally believed to be a measure of malonaldehyde ntration (Tarladgis *et al.*, 1964) and had been organoleptically correlated with ity (Zisper *et al.*, 1964).

**ulsion sausage incorporated with whey protein concentrate**

TBA number of emulsion sausages samples incorporated with whey protein ntrate with different levels (1, 2, 3 and 4%) were found to be 0.191, 0.175, 0.174 180 respectively, while control sample had TBA number as 0.179 (Table- 4.2). eatment of whey protein concentrate brought a slight change the TBA number samples. Different levels of WPC significantly ($p<0.05$) increased the TBA er of emulsion sausages samples.

**b) Emulsion sausage incorporated with whey protein isolate**

TBA number of emulsion sausages incorporated with whey protein isolate at the levels of (1, 2, 3 and 4%) were found to be 0.206, 0.221, 0.229 and 0.230 respectively, while control sample had TBA number as 0.179 (Table- 4.3). The treatment of whey protein isolate significantly ($p<0.05$) increased the TBA number of the samples. The result indicated with increasing levels of WPI increased the TBA number of emulsion sausages.

**c) Emulsion sausage incorporated with whey protein powder**

TBA number of emulsion sausages incorporated with whey protein powder at the levels of 1, 2, 3 and 4 % were found to be 0.195, 0.208, 0.202 and 0.196 respectively, while control sample had TBA number as 0.179 (Table 4.4). Addition of whey protein powder slightly increased the TBA number of emulsion sausage as compared to control sample. The treatment of whey protein powder significantly ($p<0.05$) increased the TBA number of the samples. Bhaskar *et al.*, (2009) reported the pork sausage incorporated with low, medium and high calcium milk product recorded significantly ($p<0.01$) higher mean TBA values compared to control.

**vii) Extract release volume**

Evaluation of the extract release volume (ERV) phenomenon is a rapid test for determining spoilage in meat. Extract release volume appears to have considerable possibilities for assessing the spoilage of beef (Jay and Konton 1964). It also has a highly significant correlation with water holding capacity. The procedure is based on measuring the volume of the aqueous filtrate released from slurry of meat in fixed time. The ERV decreases as spoilage progresses and no filtrate is obtained from putrid meat (Singhal *et al.*, 1997). The ERV method reveals two aspects of the spoilage mechanism. First, low temperature meat spoilage occurs in the absence of any significant breakdown of primary proteins. The second aspect of meat spoilage revealed by ERV is the increase in hydration capacity of meat proteins by some as-yet unknown mechanism (Jay *et al.*, 2005), although amino sugar complexes produced by the spoilage biota have been shown to play role (Shelef and Jay 1969a). In view of the simplicity, rapidity of performance and the apparently consistent decrease with spoilage, the ERV has proved useful for routine quality control assessment of meat.

**a) Emulsion sausage incorporated with whey protein concentrate**

In fresh condition extract release volume (ERV) of emulsion sausages incorporated with different levels (1, 2, 3 and 4%) of whey protein concentrate were found to have 34.2 ml, 35.8 ml, 36.4 ml and 36.2 ml respectively (Table- 4.2). The ERV of control sample was 33.2 ml. There is a significant difference between control sample and emulsion sausages incorporated with whey protein concentrate. The treatment of whey protein concentrate significantly ($p<0.05$) increased the ERV of the samples. It was noticed from the results of extract release volume, the emulsion sausages samples had good quality.

**b) Emulsion sausage incorporated with whey protein isolate**

Extract release volume (ERV) of emulsion sausage samples incorporated with whey protein isolate with levels of 1, 2, 3 and 4% have been presented in Table 4.3. The ERV of control sample was 33.2 ml and emulsion sausages treated with whey protein isolate with different levels (1, 2, 3 and 4%) were 37.4 ml, 38.8 ml, 35.6 ml and 36.8 ml respectively. All treated samples with different levels of WPI significantly affected the ERV of emulsion sausage samples. It was noticed from the results of extract release volume, that quality in fresh condition the emulsion sausages samples had good quality.

**c) Emulsion sausage incorporated with whey protein powder**

Extract release volume (ERV) of buffalo meat emulsion sausage incorporated with whey protein powder at different level (1, 2, 3 and 4%) have been presented in Table 4.4. In fresh condition, ERV of control sample was 33.2 ml and ERV of emulsion sausage incorporated with whey protein powder at levels of 1, 2, 3 and 4% were found to be 33.4 ml, 37.2 ml, 36.2 ml and 36.6 ml respectively. Whey protein powder a significantly ($p<0.05$) increased extract release volume of treated emulsion sausages. The emulsion sausages were found to be in good quality.

**viii) Water holding capacity**

Water holding capacity (WHC) is broadly defined as the ability of meat to retain moisture. This includes the moisture inherent to the muscle tissue as well as any fluids that may be added to the meat during further processing. Grinding of meat increase WHC by enhancing the number of polar groups available for binding with the water molecule and the water is bound better when meat in brought to gronned form (Hamm, 1970).

**a) Emulsion sausage incorporated with whey protein concentrate**

Water holding capacity of buffalo meat emulsion sausages treated with whey protein concentrate with different levels (1, 2, 3 and 4%) have been presented in Table 4.2. The WHC of control sample was 69.69% and that of emulsion sausages samples incorporated with levels of 1, 2, 3 and 4% of whey protein concentrate was found to be respectively 71.22%, 71.50%, 71.48% and 71.18% in fresh condition. An increasing trend of WHC of sausages samples was noticed with increasing the levels of WPC. Similar results was also found by Meltem and Eylem (2004), who reported addition of dairy ingredient significantly ($p<0.05$) increased WHC and emulsion satibility. A high WHC in the lean meat is a decisive factor for producing a high quality sausage (Hamm, 1970). The low ultimate pH generally increases tenderness, which is positively correlated to WHC (Govindarajulu, 2002). All treatments of WPC not significantly ($p<0.05$) affected the emulsion sausages.

**b) Emulsion sausage incorporated with whey protein isolate**

Water holding capacity of buffalo meat emulsion sausages treated with whey protein isolate with different levels (1, 2, 3 and 4%) have been presented in Table 4.3. The WHC of control sample was 69.69% and that of emulsion sausages samples incorporated with 1, 2, 3 and 4% of whey protein isolate were found to be 72.89, 72.14, 72.08 and 72.15% respectively in fresh condition. Addition of whey protein isolate in different levels (1, 2, 3 and 4%) significantly ($p<0.05$) increased of water holding capacity of emulsion sausage.

**c) Emulsion sausage incorporated with whey protein powder**

The results of evaluation of water holding capacity of buffalo meat emulsion sausage incorporated with whey protein powder with different levels (1, 2, 3 and 4%) have been presented in Table 4.4. The WHC of control sample was 69.69% and that of emulsion sausages samples incorporated with 1, 2, 3 and 4% of whey protein powder were found to be 75.33, 74.90, 75.22 and 74.60% respectively in fresh condition. Incorporation of whey protein powder at different levels (1, 2, 3 and 4%) significantly ($p<0.05$) increased the water holding capacity of emulsion sausage.

**Table 4.2:** Evaluation of physicochemical characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein concentrate.

| Sample code | Moisture content % | pH value | Ash content % | Protein content % | Fat content % | TBA No. | ERV ml | WHC % |
|---|---|---|---|---|---|---|---|---|
| Cs | 63.40± 0.011a | 6.39± 0.071e | 2.36± 0.027h | 22.58± 0.052k | 13.47± 0.015n | 0.170± 0.004q | 33.2± 0.442s | 69.69± 0.089v |
| $Swpc_1$ | 64.54± 0.012b | 6.23± 0.037fg | 2.11± 0.021hi | 23.55± 0.016m | 12.95± 0.021po | 0.191± 0.003r | 34.2± 0.836st | 71.22± 0.036vw |
| $Swpc_2$ | 64.82± 0.010c | 6.24± 0.028fg | 2.13±hi 0.028 | 23.96± 0.018mn | 12.84± 0.020p | 0.175± 0.005qr | 35.8± 0.836s | 71.50± 0.246vw |
| $Swpc_3$ | 64.70± 0.013c | 6.35± 0.081f | 2.15± 0.022i | 24.06± 0.023mn | 12.73± 0.024p | 0.174± 0.004qr | 36.4± 0.894t | 71.48± 0.225vw |
| $Swpc_4$ | 64.40± 0.010b | 6.37± 0.009f | 2.17± 0.030j | 24.10± 0.130mn | 12.69± 0.013p | 0.180± 0.006qs | 36.2± 0.836t | 71.18± 0.032vw |
| **LSD** | 0.005612 | 0.024976 | 0.012442 | 0.013472 | 0.00917 | 0.00235 | 0.37136 | 0.371361 |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$ = Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.3:** Evaluation of physicochemical characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein isolate

| Sample code | Moisture content % | pH value | Ash content % | Protein content % | Fat content % | TBA No. | ERV ml | WHC % |
|---|---|---|---|---|---|---|---|---|
| Cs | 63.40± 0.011a | 6.39± 0.071d | 2.36± 0.027g | 22.58± 0.052h | 13.47± 0.015j | 0.179± 0.004m | 33.2± 0.449o | 69.69± 0.089q |
| $Swpi_1$ | 64.21± 0.016c | 6.37± 0.097d | 2.29± 0.055g | 23.38± 0.046hi | 11.99± 0.098jk | 0.206± 0.007n | 37.4± 0.894op | 72.89± 0.086r |
| $Swpi_2$ | 64.10± 0.060bc | 6.31± 0.044e | 2.25± 0.030g | 23.84± 0.023hi | 11.73± 0.091jk | 0.221± 0.002mn | 38.8± 0.836op | 72.14± 0.023s |
| $Swpi_3$ | 64.04± 0.038bc | 6.29± 0.036ed | 2.20± 0.089g | 24.27± 0.036hi | 11.43± 0.032k | 0.229± 0.006mn | 35.6± 0.894p | 72.08± 0.027r |
| $S_{WPI4}$ | 64.08± 0.019bc | 6.28± 0.041f | 2.21± 0.027g | 24.42± 0.023hi | 11.21± 0.019k | 0.230± 0.005mn | 36.8± 0.836p | 72.15± 0.044s |
| **LSD** | 0.016262 | 0.029645 | 0.015868 | 0.017909 | 0.029641 | 0.002777 | 0.37730 | 0.02893 |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$ = Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.4:** Evaluation of physicochemical characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein powder

| Sample code | Moisture content % | pH value | Ash content % | Protein content % | Fat content % | TBA No. | ERV ml | WH % |
|---|---|---|---|---|---|---|---|---|
| Cs | 63.40± 0.011a | 6.39± 0.071d | 2.36± 0.027g | 22.58± 0.052j | 13.47± 0.015m | 0.179± 0.004q | 33.2± 0.449t | 69.6 0.08 |
| $Swpp_1$ | 65.70± 0.058b | 6.31± 0.021ef | 2.36± 0.056g | 24.90± 0.032k | 12.23± 0.051n | 0.195± 0.004r | 33.4± 0.894t | 75.3 0.09 |
| $Swpp_2$ | 64.71± 0.016bc | 6.31± 0.043ef | 2.18± 0.032h | 24.96± 0.035k | 12.34± 0.074o | 0.208± 0.006s | 37.2± 0.839v | 74.9 0.06 |
| $Swpp_3$ | 66.12± 0.021c | 6.35± 0.14f | 2.54± 0.074h | 25.12± 0.048kl | 12.20± 0.063n | 0.202± 0.002s | 36.2± 0.836wv | 75.2 0.07 |
| $Swpp_4$ | 64.96± 0.019b | 6.36± 0.017f | 2.34± 0.045i | 25.22± 0.041kl | 11.95± 0.032p | 0.196± 0.008r | 36.6± 0.547wv | 74.6 05 |
| LSD | 0.014344 | 0.018884 | 0.023751 | 0.020286 | 0.024615 | 0.00282 | 0.346575 | 0.03 |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,\ 2,\ 3,\ 4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

### 4.2.1.2 Microbiological characteristics of buffalo meat emulsion sausages incorporated with whey protein products

**i) Total plate count**

Total plate count (TPC) of the fresh emulsion sausage represents the bacterial load which in turn is indicative of quality of emulsion sausage. Ranken and Kill (1993) described the relation of bacterial population and meat products quality in the following manner: Bacterial count $10^2$ per g-excellent quality, $10^4$ per g-very good quality, $10^6$ per g-medium quality but rejection limits in many commercial contract, $10^7$ per g-spoilage begins, $10^8$ per g-meat smells, $10^9$ per g-meat becomes slimy.

**a) Emulsion sausage incorporated with whey protein concentrate**

The emulsion sausages were prepared in hygienic condition. In the present study, the samples of emulsion sausages incorporated with 1, 2, 3 and 4% of WPC had log TPC/g values 3.52, 3.60, 3.64 and 3.72 respectively. It has been indicated in Table 4.5. The TPC/g value of control sample was found to be 3.31. Different levels of whey protein concentrate significantly ($p<0.05$) increased the total plate counts of emulsion sausages.

**b) Emulsion sausage incorporated with whey protein isolate**

The emulsion sausages were prepared in hygienic condition. In the present study, the samples of emulsion sausages incorporated with 1, 2, 3 and 4% of WPI had log TPC/g values 3.69, 3.98, 3.85 and 3.80 respectively (Table 4.6). The TPC/g value of control sample was found to be 3.31. Different levels of whey protein isolate significantly ($p<0.05$) increased the total plate counts of emulsion sausages.

**c) Emulsion sausage incorporated with whey protein powder**

The emulsion sausages were prepared in hygienic condition. In present study, the samples of emulsion sausages incorporated with 1, 2, 3 and 4% of WPP had log TPC/g values 3.70, 3.93, 3.92 and 3.92 respectively (Table 4.7). The TPC/g value of control sample was found to be 3.31. Different levels of whey protein powder significantly ($p<0.05$) increased the total plate counts of ES. Incorporation of WPP with the levels of 3 and 4 % did not significantly ($p<0.05$) affect the total plat count of emulsion sausages.

**ii) Yeast and mould count**

Yeast and mold count were not detected in control the emulsion sausages samples, controlled and incorporated with whey proteins concentrate, isolate and powder in the fresh condition. The results of the study have been presented in Table 4.5, 4.6, and 4.7 respectively.

**iii) Coliform count**

Coliform count was not detected in control emulsion sausage sample and that incorporated with whey proteins concentrate, isolate and powder in the fresh condition. The results of the study have been presented in Table 4.5, 4.6, and 4.7 respectively.

**iv) Salmonella shigella**

Salmonella shigella count was not detected in emulsion sausage samples, controlled and incorporated with whey proteins concentrate, isolate and powder in the fresh condition. The results of the study have been presented in Table 4.5, 4.6, and 4.7 respectively.

**Table 4.5:** Evaluation of microbiological characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein concentrate

| Sample code | Total plate count logTPC/g | Yeast and mold count, log Y&M C/g | Coliform count | Salmonella shigella |
|---|---|---|---|---|
| **Cs** | 3.31±0.017a | ND | ND | ND |
| **$Swpc_1$** | 3.52±0.016b | ND | ND | ND |
| **$Swpc_2$** | 3.60±0.015b | ND | ND | ND |
| **$Swpc_3$** | 3.64±0.140b | ND | ND | ND |
| **$Swpc_4$** | 3.72±0.015b | ND | ND | ND |
| **LSD** | 0.030507 | | | |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%), ND= Not detected

**Table 4.6:** Evaluation of microbiological characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein isolate

| Sample code | Total plate count logTPC/g | Yeast and mold count, log Y&M C/g | Coliform count | Salmonella shigella |
|---|---|---|---|---|
| **Cs** | 3.31±0.017a | ND | ND | ND |
| **$Swpi_1$** | 3.69±0.013b | ND | ND | ND |
| **$Swpi_2$** | 3.98±0.012c | ND | ND | ND |
| **$Swpi_3$** | 3.85±0.031bc | ND | ND | ND |
| **$Swpi_4$** | 3.80±0.055bc | ND | ND | ND |
| **LSD** | 0.015048 | | | |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%), ND= Not detected

**Table 4.7:** Evaluation of microbiological characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein powder

| Sample code | Total plate count logTPC/g | Yeast and mold count, log Y&M C/g | Coliform count | Salmonella shigella |
|---|---|---|---|---|
| **Cs** | 3.31±0.017a | ND | ND | ND |
| **$Swpp_1$** | 3.70±0.082b | ND | ND | ND |
| **$Swpp_2$** | 3.93±0.032b | ND | ND | ND |
| **$Swpp_3$** | 3.92±0.030bc | ND | ND | ND |
| **$Swpp_4$** | 3.92±0.048bc | ND | ND | ND |
| **LSD** | 0.022471 | | | |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%), ND= Not detected

#### 4.2.1.3 Sensory characteristics of buffalo meat emulsion sausages incorporated with whey protein products

Sensory characteristics of fresh emulsion sausages expressed in terms of sensory attributes, namely colour, aroma, texture, taste, mouth coating, juiciness, palatability and overall acceptability. These sensory scores for different attributes of emulsion sausage samples were evaluated by a group of panel members on a nine point Hedonic scale.

**a) Emulsion sausage incorporated with whey protein concentrate**

The results of sensory evaluation have been presented in Table 4.8. The colour scores of controlled emulsion sausage were found to be 7.7. It indicated like very much condition, while the colour scores of treated sausages samples were higher in score values as compared to control sample. The score values of colour of treated sample were found in the range of 8.4-8.7. These results indicated the like very much to like extremely conditions. The score values of aroma were found to be in the range 8.3-8.5, which indicated very good condition of the treated sausages samples. The control sample had a score of 8.4 for aroma. Texture, taste, mouth coating, juiciness, palatability and overall acceptability of fresh emulsion sausage incorporated with whey protein concentrate were found to have more than eight score, while the score value of control sample for these attributes was found to be less than eight. El-Magoli *et al.*, (1996) reported that level of 4% WPC was preferred than the lower WPC levels in terms of juiciness and overall acceptability. It shows that the addition of whey protein concentrate in buffalo meat emulsion sausage significantly ($p<0.05$) affected the colour, texture, taste, mouth coating, juiciness, palatability and overall acceptability of emulsion sausages. However, whey protein concentrate incorporation did not significantly ($p<0.5$) improve the aroma of sausages. Ulu (2004) claimed that addition of 0.2% WP increased hardness of cooked meat ball compared to control samples and WP had a significant effect on the chewiness of meat balls. The study indicated that hardness and chewiness increased when whey protein was added to Frankfurters (Hughes *et al.*, 1998). Lyons *et al.*, (1999) observed that increasing concentrations of whey protein decreased flavour scores.

**b) Emulsion sausage incorporated with whey protein isolate**

The results of sensory evaluation have been presented in Table 4.9. The colour score of controlled emulsion sausage sample was found 7.7. It showed like very much condition, while the colour score of treated sausages samples were higher in score values as compared to control sample. The score values of colour of treated sample were found in the range of 8.0-8.7. It explained like very much to like extremely conditions. The score values of aroma were found to be in the range 8.0-8.1, which indicated very good condition of the treated sausages samples. The control sample had a score of 8.4 for aroma. Texture, taste, mouth coating, juiciness, palatability and overall acceptability of fresh emulsion sausage incorporated with whey protein isolate were found above eight score, while the score of control sample was found less than eight. Desmond *et al.*, (1998) suggested that the addition of whey protein and oat fibre in Low-fat beef burgers was found to be acceptable to the panelists in terms of flovour and texture compared to control beef burgers. Bhaskar *et al.*, (2009) reported the formulations of pork sausages added with high calcium milk co-precipitates recorded significantly ($p<0.01$) higher mean colour, flavour, juiciness, tenderness and overall acceptability scores than the low, medium calcium milk co-precipitates and control.

**Table 4.8:** Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein concentrate

| Samples code | | Cs | Swpc$_1$ | Swpc$_2$ | Swpc$_3$ | Swpc$_4$ | LSD |
|---|---|---|---|---|---|---|---|
| Score of Sensory attribute | **Colour** | 7.7± 0.01a | 8.7± 0.02b | 8.6± 0.01b | 8.1± 0.09c | 8.4± 0.02c | 0.022091 |
| | **Aroma** | 8.4± 0.13d | 8.4± 0.25d | 8.4± 0.15d | 8.5± 0.20e | 8.3± 0.26f | 0.098678 |
| | **Texture** | 7.5± 0.07g | 8.1± 0.14g | 8.5± 0.07gh | 8.4± 0.07gh | 8.7± 0.01h | 0.045726 |
| | **Taste** | 7.9± 0.08i | 8.1± 0.08j | 8.6± 0.07jk | 8.7± 0.05jk | 8.3± 0.13j | 0.042184 |
| | **Mouth coating** | 8.1± 0.14k | 7.9± 0.22m | 8.5± 0.17n | 8.2± 0.13mn | 8.2± 0.17mn | 0.082771 |
| | **Juiciness** | 7.7± 0.14p | 8.3± 0.10q | 8.7± 0.08q | 8.3± 0.13qr | 8.2± 0.44qr | 0.106926 |
| | **Palatability** | 7.2± 0.17s | 7.8± 0.21st | 8.3±0.2 6t | 8.2± 0.20tv | 8.2± 0.17tv | 0.099378 |
| | **Overall acceptability** | 7.5± 0.25w | 8.1± 0.22x | 8.5± 0.24y | 8.4± 0.26y | 8.0± 0.50x | 0.15268 |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpc$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**c) Emulsion sausage incorporated with whey protein powder**

The results of sensory evaluation have been presented in Table 4.10. The colour score of controlled emulsion sausage sample was found 7.7. It showed like very much condition, while the colour score of treated sausages samples were higher in score values as compared to control sample. The score values of colour of treated sample were found in the range of 7.9-8.3. It explained like very much to like extremely conditions. The score values of aroma were found to be in the range 8.2-8.4, which indicated very good condition of the treated sausages samples. The control sample had a score of 8.4 for aroma. Texture, taste, mouth coating, juiciness, palatability and overall acceptability of fresh emulsion sausage incorporated with whey protein powder were found above eight score, while the score of control sample was found less than eight. Ulu (2004) quoted that addition of 0.2% whey protein increased hardness of cooked meat ball compared to control samples and treated samples had a significant effect on the chewiness of meatballs. Likewise, another study showed that hardness and chewiness increased when WP was added to Frankfurters (Hughes *et al.*, 1998). Andic *et al.*, (2010) reported that addition of 1 and 2% whey protein and skim milk powder made no significant ($p<0.01$) differences in appearance, interior colour, juiciness and flavour scores of patties. Holland (1984) claimed that additions of whey powder to comminuted beef patties increased flavour scores.

**Table 4.9:** Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of whey protein isolate

| Samples code | | Cs | $Swpi_1$ | $Swpi_2$ | $Swpi_3$ | $Swpi_4$ | LSD |
|---|---|---|---|---|---|---|---|
| Score of Sensory attribute | **Colour** | 7.7± 0.01a | 8.0± 0.070b | 8.6± 0.054c | 8.7± 0.89c | 8.4± 0.089d | 0.032764 |
| | **Aroma** | 8.4± 0.13e | 8.0± 0.089ef | 8.0± 0.087ef | 8.1± 0.089ef | 8.1± 0.083ef | 0.046032 |
| | **Texture** | 7.5± 0.07g | 8.6± 0.054hi | 8.3± 0.089i | 8.4± 0.083hi | 8.2± 0.054hi | 0.03401 |
| | **Taste** | 7.9± 0.08j | 8.3± 0.089jk | 8.2± 0.054k | 8.2± 0.044k | 8.1± 0.070k | 0.03401 |
| | **Mouth coating** | 8.1± 0.14m | 8.1± 0.89m | 8.0± 0.054m | 7.9± 0.083m | 8.2± 0.070m | 0.044743 |
| | **Juiciness** | 7.7± 0.14n | 7.6± 0.070n | 7.6± 0.089n | 8.0± 0.089o | 7.8± 0.054no | 0.044243 |
| | **Palatability** | 7.2± 0.17p | 8.5± 0.070pq | 8.2± 0.070q | 8.2± 0.083q | 8.3± 0.083q | 0.049013 |
| | **Overall acceptability** | 7.5± 0.25r | 8.0± 0.044s | 8.0± 0.054s | 8.1± 0.054s | 7.9± 0.083s | 0.066028 |

Values are mean of five replicates ±SD; LSD = Least significance difference, Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.10:** Evaluation of sensory characteristics of fresh buffalo meat emulsion sausage incorporated with different levels of protein powder

| Samples code | | Cs | $Swpp_1$ | $Swpp_2$ | $Swpp_3$ | $Swpp_4$ | LSD |
|---|---|---|---|---|---|---|---|
| Score of Sensory attribute | Colour | 7.7± 0.011a | 8.3± 0.023b | 8.3± 0.014b | 8.1± 0.018c | 7.9± 0.016d | 0.00825 |
| | Aroma | 8.4± 0.013e | 8.4± 0.012e | 8.4± 0.019e | 8.3± 0.016ef | 8.2± 0.016ef | 0.028361 |
| | Texture | 7.5± 0.070g | 8.3± 0.021h | 8.4± 0.020hi | 8.4± 0.016hi | 8.4± 0.020hi | 0.04677 |
| | Taste | 7.9± 0.089j | 8.5± 0.070k | 8.0± 0.083l | 8.3± 0.089ml | 8.4± 0.083m | 0.039459 |
| | Mouth coating | 8.1± 0.148n | 7.9± 0.070n | 7.6± 0.089no | 7.9± 0.083n | 8.1± 0.083n | 0.046689 |
| | Juiciness | 7.7± 0.141p | 8.1± 0.089p | 7.9± 0.054pq | 8.0± 0.054pq | 8.0± 0.089pq | 0.043226 |
| | Palatability | 7.2± 0.173r | 8.5± 0.007s | 8.6± 0.018s | 8.4± 0.021s | 8.3± 0.005s | 0.038954 |
| | Overall acceptability | 7.5± 0.258w | 8.2± 0.021wz | 8.1± 0.011wz | 8.2± 0.018wz | 8.0± 0.058wz | 0.062366 |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), LSD = Least significance difference, Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

### 4.2.1.4 Instrumental measurement of colour

The instrument describes the colour in three dimensional system indicating value, hue and chroma (L, a and b). Value refers to lightness/darkness and it distinguishes light colour from dark or white from black. Maximum brightness is reported as 100. Hue is the colour attribute by which an object/food is judged to red, yellow, green, blue and so forth. It actually describes the shade of red/ yellow/ green/ blue. The four basic colours are given max numerical value 60. Chroma refers to vividness, depth, purity and saturation of a particular colour. It describes how much a particular object/ foundation is yellow/red/ green/ blue.

#### a) Emulsion sausage incorporated with whey protein concentrate

Table 4.11- presented the results of colour evaluation of emulsion sausages by Hunter Lab. Colour measurement of buffalo meat emulsion sausage prepared by different levels of whey protein concentrate has done by Hunter Lab. The measurement of colour of samples was done in fresh condition. The numerical value of 'L' sample in fresh condition was found to be in the range of 29.50%-33.25%. Thus sample had 33.25% of maximum lightness as compared to 66.75% darkness.

The sample of fresh emulsion sausages was grayish brown colour in appearance. Hue values were found in the range 3.9-4.92. This shows that maximum red colour was 4.92 as compared to yellow colour 'Chroma' value were found in the range of 8.4-9.22. Incorporation of whey protein concentrate was added at the levels of 1, 2, 3 and 4% in emulsion sausages caused slight decrease in L* and b* values of colour and increased in a* value compare to control sample. Atughonu *et al.*, (1998) asserted a significant increase in L* values and a decrease in a* value when 3.5% whey protein concentrate was added to regular beef and pork frankfurters.

**b) Emulsion sausage incorporated with whey protein isolate**

Table 4.12 present the results of colour evaluation of emulsion sausages by Hunter Lab. The measurement of colour of samples was done in fresh condition. The numerical values of 'L' sample of emulsion sausages incorporated with levels of 1-4% in fresh condition were found in the range of 22.31% - 25.07%. Thus sample had 25.7% of maximum lightness as compared to 74.93% darkness. The sample of fresh emulsion sausages were grayish brown colour in appearance. Hue values were found in the range of 4.26-6.08. This showed that maximum red colour was 6.08 as compared to yellow colour 'Chroma' value were found in the range of 4.72-7.45.

**c) Emulsion sausage incorporated with whey protein powder**

Table 4.13 present the results of colour evaluation of emulsion sausages by Hunter Lab. The measurement of colour of samples was done in fresh condition. The numerical value of 'L' sample in fresh condition incorporated with whey protein powder found in the range of 20.49 - 20.76%. Thus sample had 20.76% of maximum lightness as compared to 79.33% darkness. The sample of fresh emulsion sausages was grayish brown colour in appearance. Hue values were found in the range 3.35 - 4.61. This showed that maximum red colour was 4.61 as compared to yellow colour 'Chroma' value were found in the range of 4.96 - 6.09. Meltem (2006) reported the fat level (20%) and Whey protein significantly affected L* values of cooked meat balls ($p<0.05$). Increasing the fat level in formulation resulted lower a* values but whey protein had no effect on a* values of meatballs. Neither the fat level nor the whey protein level affected b* values of samples ($p>0.05$).

**Table-4.11** Effect of different levels whey protein concentrate incorporation on color of buffalo meat emulsion sausage as measured by Hunter lab

| Samples code | | Cs | $Swpc_1$ | $Swpc_2$ | $Swpc_3$ | $Swpc_4$ |
|---|---|---|---|---|---|---|
| Colour measurement | L | 33.25 | 31.23 | 30.28 | 29.50 | 30.37 |
| | a | 3.9 | 4.92 | 4.91 | 4.36 | 4.53 |
| | b | 9.22 | 8.76 | 8.85 | 8.4 | 8.8 |

Cs = Control sample, $Swpc_{1, 2, 3, 4}$ = Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table-4.12** Effect of different levels whey protein isolate incorporation on color of buffalo meat emulsion sausage as measured by Hunter lab

| Samples code | | Cs | $Swpi_1$ | $Swpi_2$ | $Swpi_3$ | $Swpi_4$ |
|---|---|---|---|---|---|---|
| Colour measurement | L | 33.25 | 23.17 | 22.31 | 22.72 | 25.07 |
| | A | 3.9 | 4.60 | 6.08 | 4.26 | 5.24 |
| | B | 9.22 | 7.72 | 6.17 | 6.57 | 7.45 |

Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table-4.13** Effect of different levels whey protein powder incorporation on color of buffalo meat emulsion sausage as measured by Hunter lab

| Samples code | | Cs | $Swpp_1$ | $Swpp_2$ | $Swpp_3$ | $Swpp_4$ |
|---|---|---|---|---|---|---|
| Colour measurement | L | 33.25 | 20.49 | 20.57 | 19.33 | 20.76 |
| | a | 3.9 | 4.61 | 3.35 | 3.99 | 3.85 |
| | b | 9.22 | 6.09 | 4.96 | 5.2 | 5.41 |

Cs = Control sample, $Swpp_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

### 4.2.2 Physico-chemical characteristic of buffalo meat emulsion sausage during refrigerated storage 0°C

#### 4.2.2.1 Moisture content

Moisture content of emulsion sausages is important property, which related to the quality and shelf life of the product. Emulsion sausage has high moisture content. It is advisable to keep the product under refrigerated condition. The shelf life of emulsion sausage controlled and treated with whey protein concentrate, isolate and whey protein powder have been determined under refrigerated condition (0°C).

**a) Emulsion sausage incorporated with whey protein concentrate**

The moisture contents of emulsion sausages (ES) control and incorporated with different levels (1, 2, 3 and 4%) of whey protein concentrate (WPC) have been presented in Table 4.14. Moisture content of these samples was found to be in between 64.40% and 64.82% (on wet basis) in fresh condition. Moisture content of control sample was 63.40% in fresh condition. It indicated that the moisture content of control sample was lower as compared to incorporated whey protein concentrate products. During refrigerated storage, moisture content of these samples significantly ($p<0.05$) decreased. The moisture contents of samples were found in the range of 60.28 - 60.73% on the 25th day of storage (Fig. 4.1). These finding are in agreement with Serdaroğlu and Özsümer (2003), which indicated that the addition of whey powder caused a slight increase in moisture content and the range of moisture content was 62.0% in cooked beef sausages. The ANOVA results showed that the sausage samples incorporated with different levels (1, 2, 3 and 4%) of WPC had significant ($p<0.05$) effect on the moisture content (Table 4.15).

Fig 4.2 shows the linear regression of moisture content of emulsion sausage incorporated with whey protein concentrate at the levels of 1, 2, 3 and 4% of during refrigerated storage at 0° C. The negative sign in the coefficients of x explains that there was constant decrease of moisture content during refrigerated storage. The value of correlation coefficient ($R^2$) of all samples was near to 1 and so almost perfect relation existed between storage period and moisture content.

**b) Emulsion sausage incorporated with whey protein isolate**

The moisture contents of emulsion sausage (ES) samples incorporated with different levels of whey protein isolate (WPI) have been presented in Table 4.16. The initial moisture contents of sausage samples with the levels of 1, 2, 3 and 4% whey protein isolate were respectively 64.21, 64.01, 64.04 and 64.08%. The moisture content of control sample was 63.40%. It showed that treatment of WPI increased the moisture content of emulsion sausage. With increasing levels of whey proteins isolate moisture contents of emulsion sausage samples were found to increase when it compared to control sample. Fig 4.3 shows the moisture content treated and controlled samples with respect to storage time. The ANOVA results (Table 4.17) indicated different levels (1, 2, 3 and 4%) of whey protein isolate had significant ($p<0.05$) affect for moisture content of emulsion sausages samples. At the end of 25th day of refrigerated storage, it was found that the moisture contents of control and treated samples with (1, 2, 3 and 4%) of WPI were respectively 59.47 5%, 59.82%, 59.73%, 59.91% and 59.90%. This indicated that there were slight increases in moisture content of treated sample as compared to control sample. However the end results showed a fluctuating pattern of moisture contents among the treated samples.

Fig 4.4 demonstrates the linear regression of moisture content of emulsion sausage samples during refrigerated storage at 0°C. The negative sign in the coefficients of x explains that that there was constant decrease in moisture content during refrigerated storage. The correlation coefficient values explain the correlation between moisture content and storage period (days). Decreasing nature of moisture content with storage days time was in fact perfect at $R^2=1$. The values of $R^2$ for all five samples were between 0.9541 to 0.989, which proofs that correlations are almost perfect and the graph may be approximated to a straight line. The moisture loss during storage was may due to permissibly of low-density polyethylene (LDPE) film packaging.

**c) Emulsion sausage incorporated with whey protein powder**

Table 4.18 presents the results of moisture content of buffalo meat emulsion sausage samples prepared with 1, 2, 3 and 4% of whey protein powder (WPP) under refrigerated storage at 0°C. The moisture content of all samples of emulsion sausages were found in the range of 64.71-66.12% in fresh condition. Different percentage of WPP significantly ($p<0.05$) increased the moisture content of emulsion sausage samples (Table 4.19). Andic *et al.*, (2010) stated that the moisture content of cooked patties treated with whey powder and skim milk powder was significantly ($p<0.05$) higher than those of control samples. These results matched with observation of Hung and Zayas (1992). Serdaroğlu (2006) explained that the addition of 2% and 4% whey powder decreased the moisture of meatball standardized to 5% fat level, while increased the moisture content of samples standardized to 10-20% fat level. Addition of whey powder slightly altered the moisture content of raw meatballs due to the increase in dry matter in the meatball formulation (Meltem, 2006). Similar results were found by Desmond *et al.*, (1998). Fig 4.3 shows the moisture content treated samples and controlled sample with respect to storage period. A moderate decreasing pattern of moisture contents of emulsion sausages were found during refrigerated storage. The moisture content of control sample was found as 59.47% while sausage samples were found to be 60.83, 59.76, 61.14 and 59.87% respectively that moisture content of whey protein power treated with different levels (1, 2, 3 and 4%) on the $25^{th}$ day of storage. The decrease in moisture content was due to the evaporation of moisture through the permeable packaging film. Bhaskar *et al.*, (2009) asserted the overall percent moisture content of pork sausages had decreased significantly ($p<0.01$) with the storage period under refrigerated storage conditions. It was found that the use of different levels of whey protein powder significantly ($p<0.05$)

decreased moisture content of emulsion sausages samples. The highest increase in moisture content was noted in the sample incorporated with 3% whey powder.

Fig 4.6 shows the linear regression of moisture content of emulsion sausage incorporated with whey protein powder at the levels of 1, 2, 3 and 4% of during refrigerated storage at 0°C. The equations of regression and correlation coefficient ($R^2$) have been shown in the regression graph. Values of $R^2$ were very close to 1 and hence the perfect linear relation was exhibited for consistent reduction in moisture content during refrigerated storage.

**Table 4.14:** Effect of refrigerated storage (0°C) on moisture content of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample code | Moisture content% | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 63.40± 0.011a | 63.16± 0.066 | 62.19± 0.035 | 61.81± 0.069 | 60.83± 0.045 | 59.47± 0.036f |
| **$Swpc_1$** | 64.54± 0.012b | 63.94± 0.033 | 62.54± 0.080 | 62.08± 0.040 | 61.79± 0.068 | 60.67± 0.071g |
| **$Swpc_2$** | 64.82± 0.010c | 63.79± 0.053 | 63.13± 0.044 | 62.19± 0.045 | 61.82± 0.060 | 60.82± 0.059h |
| **$Swpc_3$** | 64.70± 0.013d | 64.12± 0.035 | 63.25± 0.081 | 62.47± 0.073 | 61.73± 0.070 | 60.28± 0.068i |
| **$Swpc_4$** | 64.40± 0.010e | 64.11± 0.042 | 63.59± 0.053 | 62.52± 0.055 | 61.23± 0.036 | 60.73± 0.051j |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.15:** ANOVA of moisture content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 302.996 | 2.03353 | | |
| **Replicate** | 4 | 0.003736 | 0.000934 | 0.302166 | 2.46 |
| **FA** | 4 | 20.38034 | 5.095085 | 1648.488 | 2.46 |
| **FB** | 5 | 275.0039 | 55.00078 | 17795.21 | 2.3 |
| **Comb(A*B)** | 29 | 7.281065 | 0.251071 | 81.23277 | 1.63 |
| **Error/Res** | 107 | 0.330712 | 0.003091 | | |
| **LSD** | 0.069619 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.1:** Effect of refrigerated storage (0°C) on moisture content of emulsion sausages incorporated with different levels of whey protein concentrate

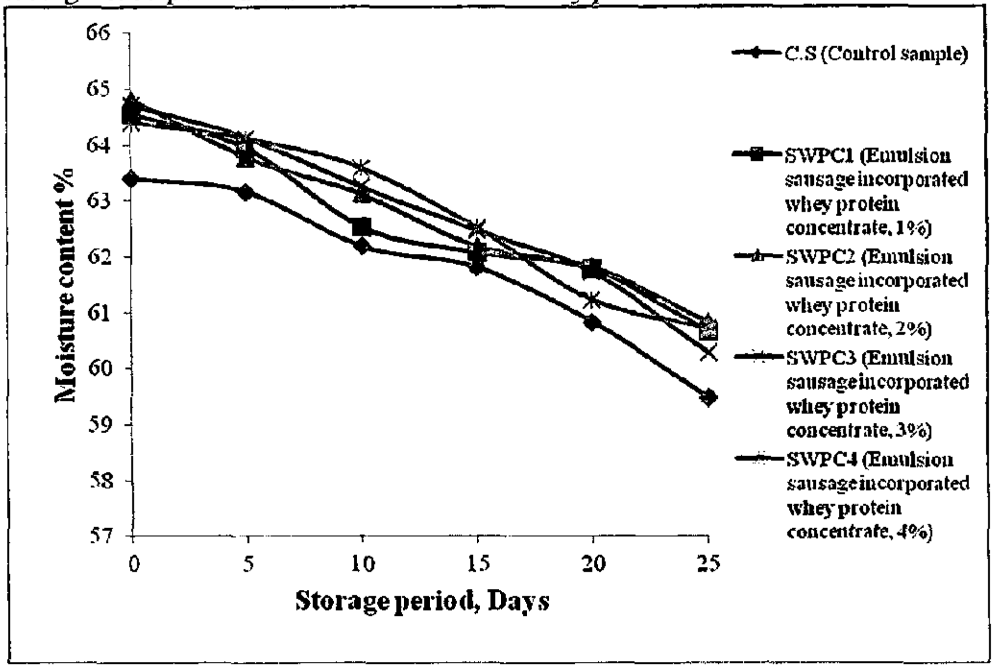
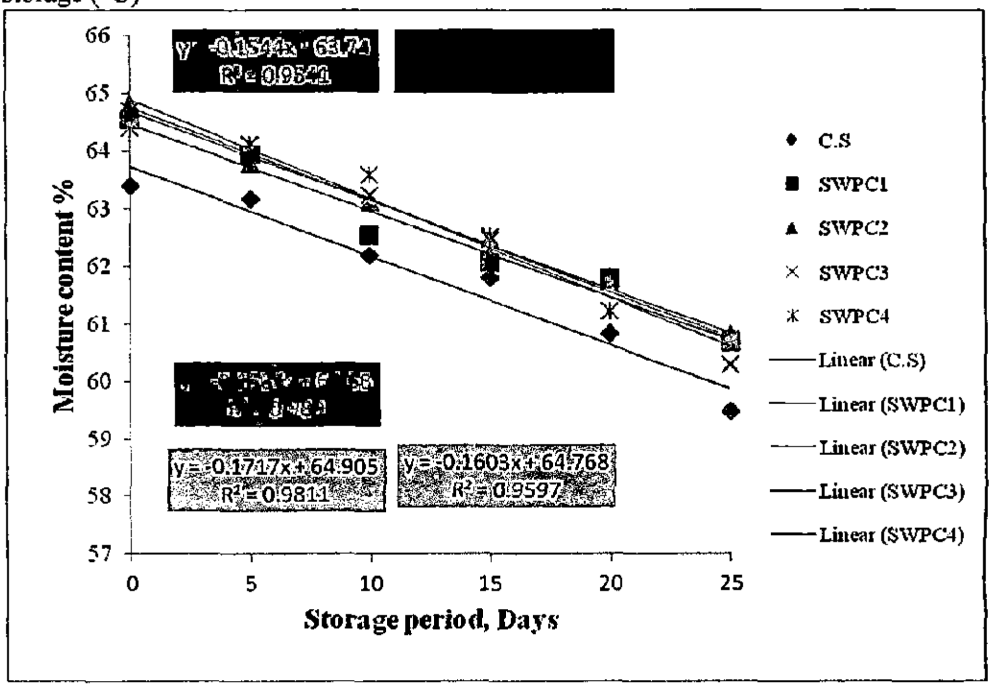



**Figure 4.2:** Regression analysis of moisture content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (°C)



**ble 4.16:** Effect of refrigerated storage (0°C) on moisture content of emulsion sages incorporated with different levels of whey protein isolate

| mple ode | Moisture content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 63.40± 0.011a | 62.16± 0.066 | 62.19± 0.038 | 61.81± 0.069 | 61.81± 0.047 | 59.47± 0.036e |
| **wpi$_1$** | 64.21± 0.016b | 63.12± 0.033 | 61.93± 0.019 | 61.93± 0.019 | 60.88± 0.022 | 59.82± 0.019f |
| **wpi$_2$** | 64.10± 0.060c | 62.45± 0.041 | 61.08± 0.050 | 60.66± 0.030 | 60.23± 0.047 | 59.73± 0.022g |
| **wpi$_3$** | 64.04± 0.038d | 63.74± 0.065 | 62.17± 0.045 | 61.12± 0.013 | 60.94± 0.026 | 59.91± 0.019h |
| **wpi$_4$** | 64.08± 0.019d | 62.71± 0.038 | 62.61± 0.068 | 61.36± 0.043 | 60.16±0 .024 | 59.90± 0.023h |

ies are mean of five replicates ±SD; Means with different letters in a column differ significantly ).05), Cs = Control sample, Swpi$_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of y protein isolate (1, 2, 3 and 4%)

**ble 4.17:** ANOVA of moisture content of emulsion sausages incorporated with erent levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 318.2035 | 2.135594 | | |
| **eplicate** | 4 | 0.007289 | 0.001822 | 1.001059 | 2.46 |
| **FA** | 4 | 1.798101 | 0.449525 | 246.9427 | 2.46 |
| **FB** | 5 | 301.2856 | 60.25713 | 33101.72 | 2.3 |
| **mb(A*B)** | 29 | 14.92495 | 0.514653 | 282.7202 | 1.63 |
| **ror/Res** | 107 | 0.194779 | 0.00182 | | |
| **LSD** | 0.053429 | | | | |

= Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, ) = Least significance difference, FA = Different levels of whey protein incorporated, = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.3:** Effect of refrigerated storage (0°C) on moisture content of emulsion sausages incorporated with different levels of whey protein isolate

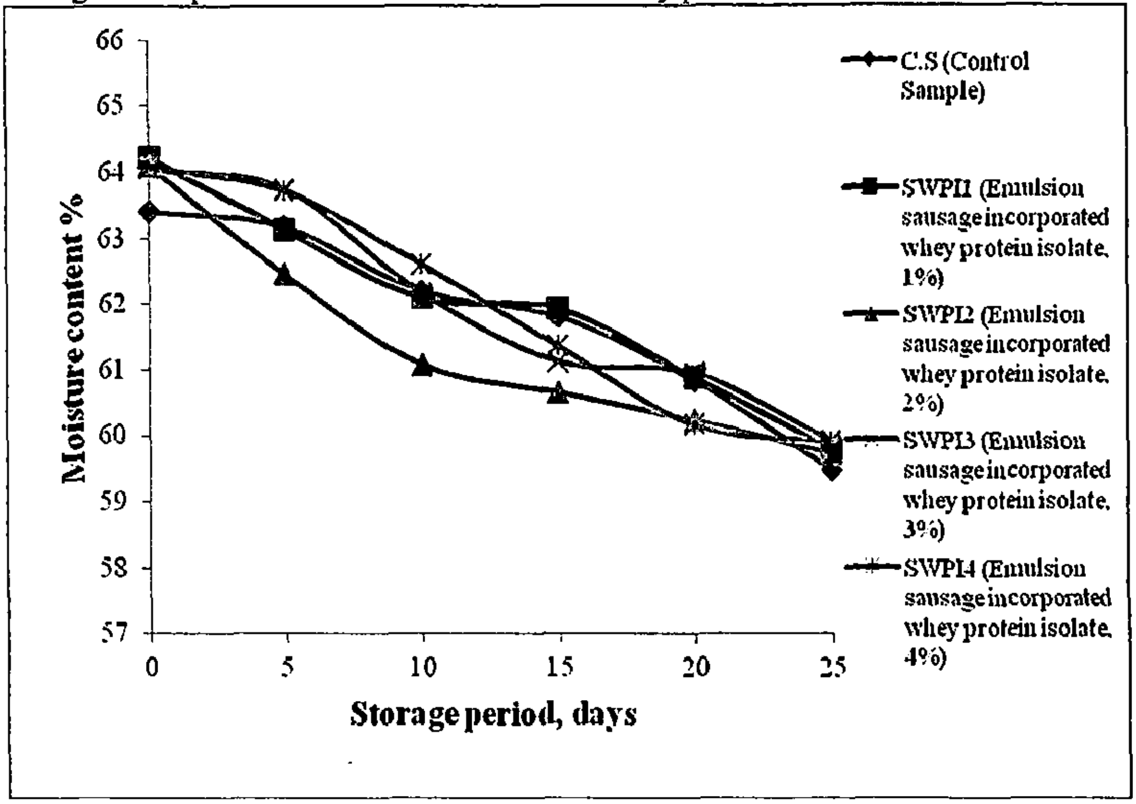



**Figure 4.4:** Regression analysis of moisture content of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

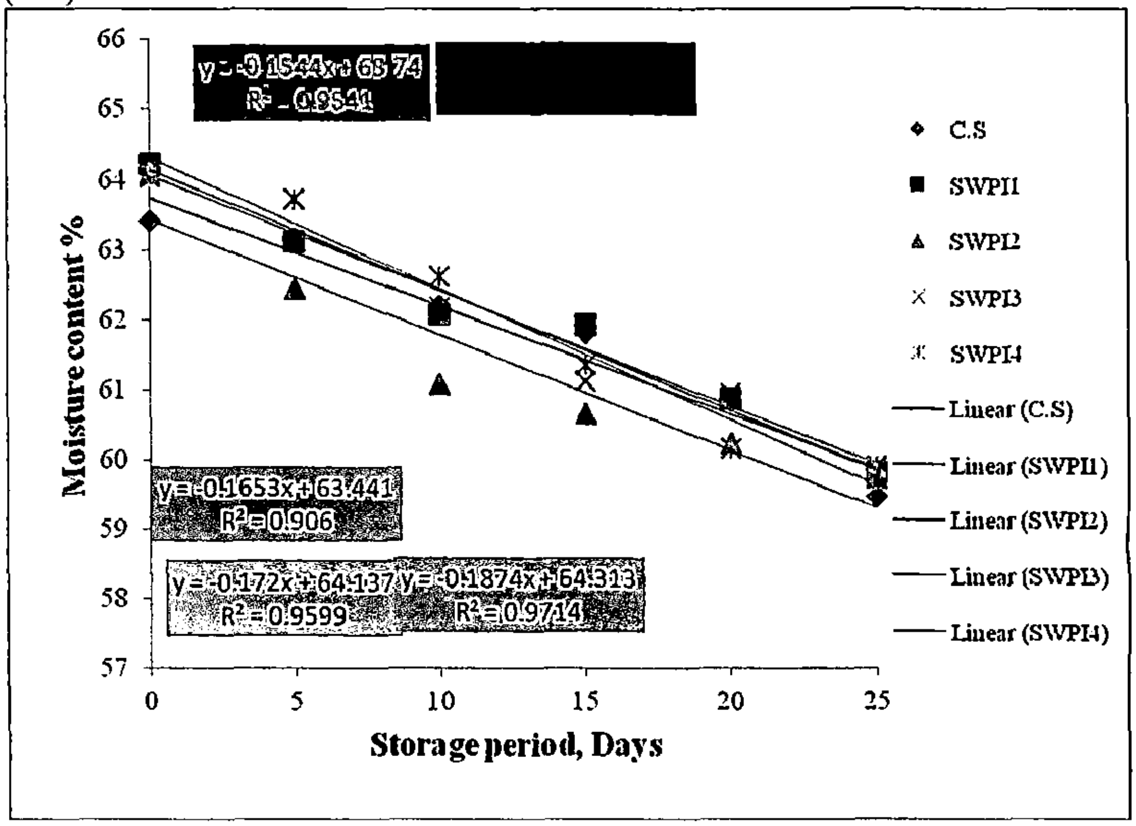


**Table 4.18:** Effect of refrigerated storage (0°C) on moisture content of emulsion sausages incorporated with different levels of whey protein powder

| Sample code | Moisture content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 63.40± 0.011a | 63.16± 0.066 | 62.19± 0.038 | 61.81± 0.069 | 60.83± 0.047 | 59.47± 0.036g |
| **$Swpp_1$** | 65.70± 0.058b | 64.31± 0.025 | 63.41± 0.030 | 62.00± 0.014 | 61.83± 0.031 | 60.83± 0.023h |
| **$Swpp_2$** | 64.71± 0.016c | 63.83± 0.057 | 62.08± 0.040 | 61.88± 0.057 | 60.23± 0.028 | 59.76± 0.040i |
| **$Swpp_3$** | 66.12± 0.021e | 65.94± 0.040 | 64.06± 0.049 | 63.87± 0.037 | 62.83± 0.054 | 61.14± 0.062j |
| **$Swpp_4$** | 64.91± 0.019f | 63.36± 0.030 | 62.34± 0.045 | 61.16± 0.058 | 60.69± 0.034 | 59.87± 0.76k |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Sausage Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.19:** ANOVA of moisture content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 509.5673 | 3.419915 | | |
| **Replicate** | 4 | 0.060509 | 0.015127 | 1.208955 | 2.46 |
| **FA** | 4 | 101.6219 | 25.40548 | 2030.376 | 2.46 |
| **FB** | 5 | 391.8158 | 78.36315 | 6262.69 | 2.3 |
| **Comb(A*B)** | 29 | 14.79077 | 0.510027 | 40.76071 | 1.63 |
| **Error/Res** | 107 | 1.338859 | 0.012513 | | |
| **LSD** | 0.140078 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.5:** Effect of refrigerated storage (0°C) on moisture content of emulsion sausages incorporated with different levels of whey protein powder

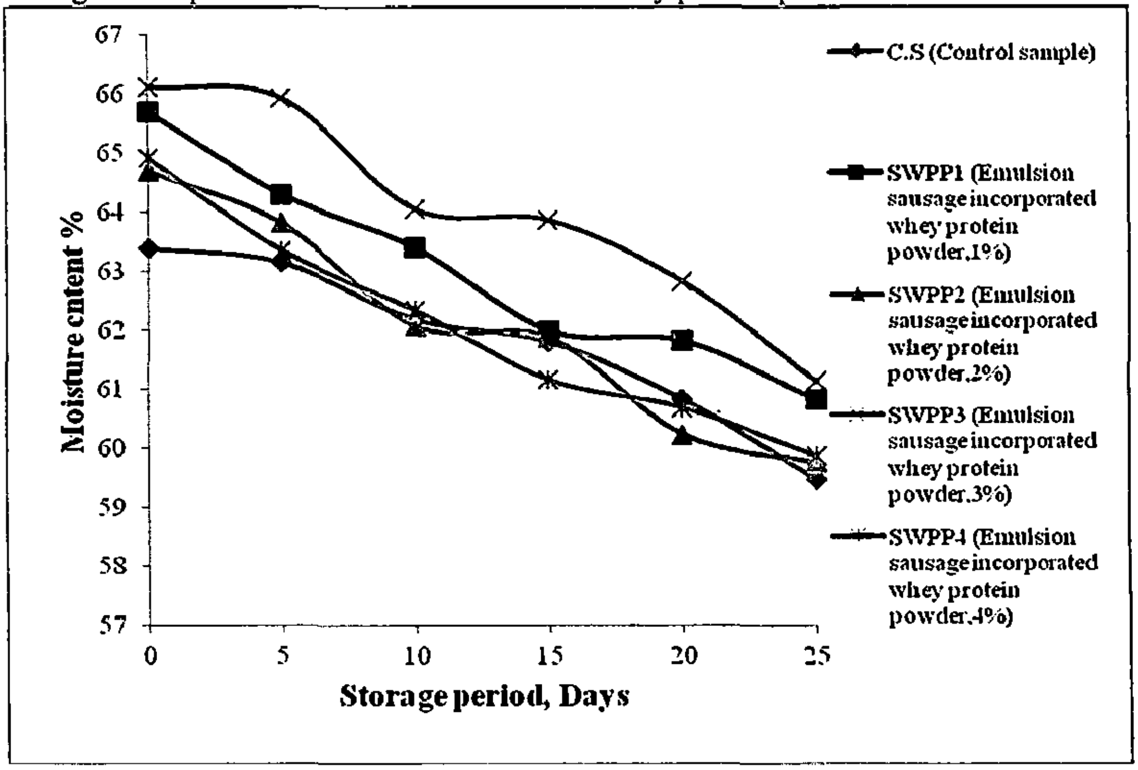
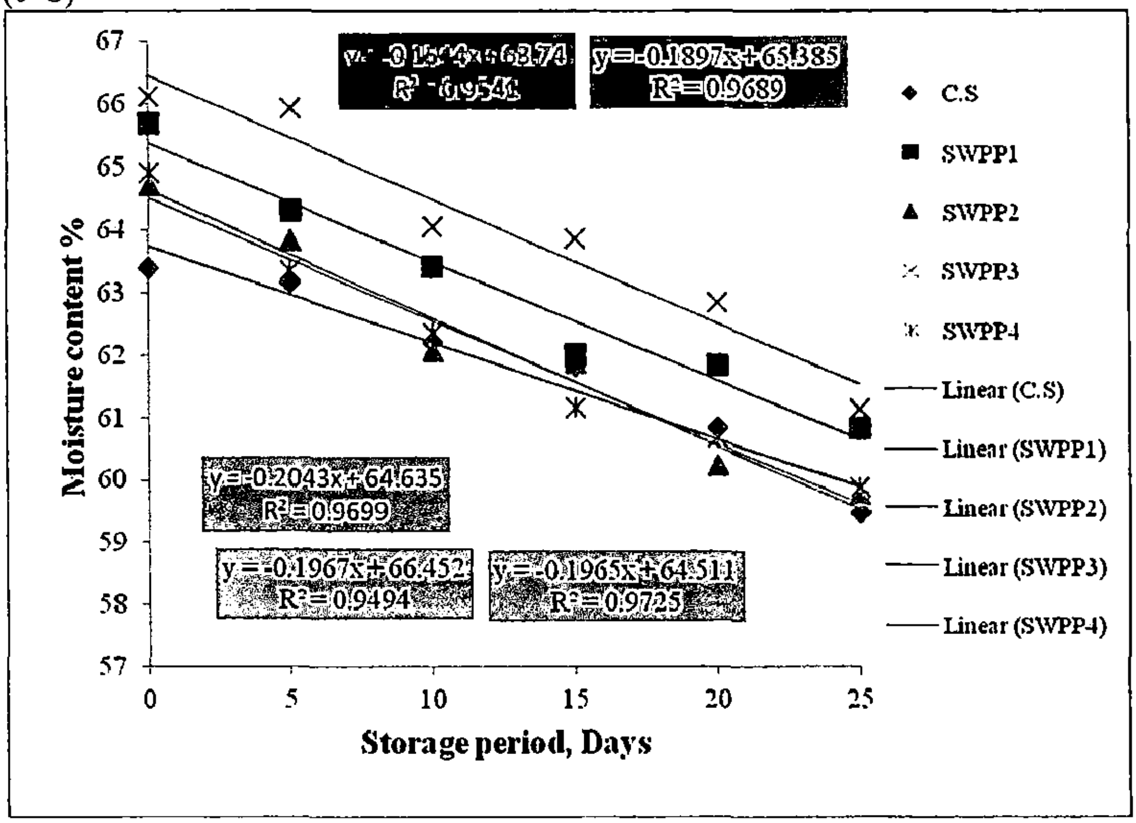



**Figure 4.6:** Regression analysis of moisture content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.2.2 pH values

pH of meat changes during postmortem glycolysis and it decreases to lowest value stage (common as ultimate) pH stage. pH plays important role in preservation. Meat is a source of bacterial and mold spoilage. Sausage contains ingredients like spices, condiments, fat and antioxidant which offer with in microbial growth.

**a) Emulsion sausage incorporated with whey protein concentrate**

The results of pH have been presented in Table 4.20. Sausage samples had pH values between 6.23-6.39 in fresh condition. pH of emulsion sausage is evaluated before and during refrigerated storage at 0°C after every five days. Fig 4.7 shows the pH profile of emulsion sausages samples during refrigerated storage (0°C). Different levels of whey protein concentrate (1, 2, 3 and 4%) made a slight change the pH value of sausage samples. During refrigerated storage at 0°C, pH values were found to decrease consistently, and refrigerated storage significantly ($p<0.05$) reduced the pH value of emulsion sausages incorporated with different levels whey protein concentrate. The analysis of variance (ANOVA Table 4.21) demonstrated that the use of different levels of WPC significantly ($p<0.05$) affected for the pH of emulsion sausages sample. At the end of 25 days of storage, pH values were found to be in the range of 5.71-5.78. Similar result found by Kala *et al.*, (2007), this study showed that the change in pH of chicken emulsion patties occurred during refrigerated storage at 4±1°C. The pH value of control sample was 5.83 at end of 25$^{th}$ day.

The equation of regression lines and correlation coefficients of emulsion sausages treated with whey protein concentrate with different levels have been shown on the regression graph (Fig 4.8). The negative sign in the coefficients of x explains that there was a slight decrease of pH during refrigerated storage. The values of $R^2$ for all samples at different levels of whey protein concentrate 1, 2, 3, 4% and control sample were 0.9806, 0.9976, 0.9981, 0.9945 and 0.986 respectively. The values of $R^2$ were near to 1, thus the graph may be approximated to a straight line and linear relation pH and storage period well fits a linear model.

**b) Emulsion sausage incorporated with whey protein isolate**

Table 4.22 presents the results of pH values of emulsion sausage incorporated with whey protein isolate at different levels (1, 2, 3 and 4 %) and control sample. The initial pH of emulsion sausage samples was found to be 6.36, 6.31, 6.29, 6.28 and 6.39 respectively. Additions of WPI led to a slight change of pH values of samples. During refrigerated storage at 0°C, pH values were found to decrease consistently and pH measurement was carried out every after 5th day, till the end of shelf life. Fig 4.9 shows the pH profile of emulsion sausages samples during refrigerated storage (0°C). The ANOVA result (4.23) indicated refrigerated storage significantly ($p<0.05$) reduced the pH value of emulsion sausages incorporated with whey protein isolate with levels 1, 2, 3 and 4%. At the end of 25 days of storage, pH values were found to be 5.90, 5.69, 5.68 and 5.69 for treated samples. The pH value of control sample was 5.83 at the end of 25 days. The ANOVA results showed that the use of different level of whey protein isolate did not significantly ($p<0.05$) affected the pH value of emulsion sausages samples.

Fig 4.10 presents the equation of regression lines and correlation coefficients of emulsion sausages incorporated with whey protein isolate with levels 1, 2, 3 and 4%. The negative sign in the coefficients of x explains that there was consistent decrease in pH values during refrigerated storage. The correlation coefficient values explain that correlation between pH value and storage period (days). The decreasing nature of pH value with storage time (days) was in fact perfect at $R^2=1$ for linear system. The values of $R^2$ for all samples were in between 0.9447- 0.986 (Fig. 4.10).

**c) Emulsion sausage incorporated with whey protein powder**

pH values of the all samples were found between 6.31-6.42 just after preparation of samples (Table 4.24). Different levels of whey protein powder significantly ($p<0.05$) decreased the pH value of emulsion sausages. These results are in agreement with the findings by Ensoy *et al.,* (2010). During refrigerated storage at 0°C, pH values of samples were found significantly ($p<0.05$) decrease. At the end of 25 days of storage pH values were between 5.668-5.894. Fig 4.11 shows the pH profile of emulsion sausages samples during refrigerated storage (0°C). Increasing the levels of whey protein powder from 1, 2, 3 and 4% made a slight decrease the pH values of all samples. The ANOVA (Table 4.25) results showed that the use of different level (1, 2, 3 and 4%) of WPP a significant ($p<0.05$) for pH value of emulsion sausages samples.

The equation of regression lines and correlation coefficient of all samples with different levels (1, 2, 3 and 4%) of whey protein powder have been shown on the regression graph (Fig 4.12). The negative sign in the coefficients of x explains that there was constant decrease of pH during storage. The values of $R^2$ for all samples at different levels (1-4%) of whey protein powder were between 0.9194–0986. The values of $R^2$ were near to 1, thus the graph may be approximated to a straight line and linear relation between storage period and pH values well fits in a linear system.

**Table 4.20:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample code | pH value | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 6.390± 0.071a | 6.286± 0.009 | 6.167± 0.016 | 6.011± 0.007 | 5.976± 0.004 | 5.834± 0.009c |
| **Swpc₁** | 6.236± 0.037b | 6.180± 0.023 | 6.054± 0.028 | 5.989± 0.008 | 5.815± 0.003 | 5.750± 0.021d |
| **Swpc₂** | 6.242± 0.028b | 6.124± 0.023 | 6.046± 0.018 | 5.915± 0.003 | 5.818± 0.005 | 5.711± 0.004d |
| **Swpc₃** | 6.358± 0.081a | 6.260± 0.048 | 6.136± 0.020 | 6.010± 0.011 | 5.881± 0.011 | 5.783± 0.048d |
| **Swpc₄** | 6.378± 0.009a | 6.261± 0.033 | 6.154± 0.024 | 5.990± 0.002 | 5.857± 0.006 | 5.769± 0.038d |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.21:** ANOVA of pH value of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 6.100954 | 0.040946 | | |
| **Replicate** | 4 | 0.008967 | 0.002242 | 2.245783 | 2.46 |
| **FA** | 4 | 0.359922 | 0.08998 | 90.14342 | 2.46 |
| **FB** | 5 | 5.563511 | 1.112702 | 1114.718 | 2.3 |
| **Comb(A*B)** | 29 | 0.070715 | 0.002438 | 2.44285 | 1.63 |
| **Error/Res** | 107 | 0.106807 | 0.000998 | | |
| **LSD** | 0.039564 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.7:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein concentrate

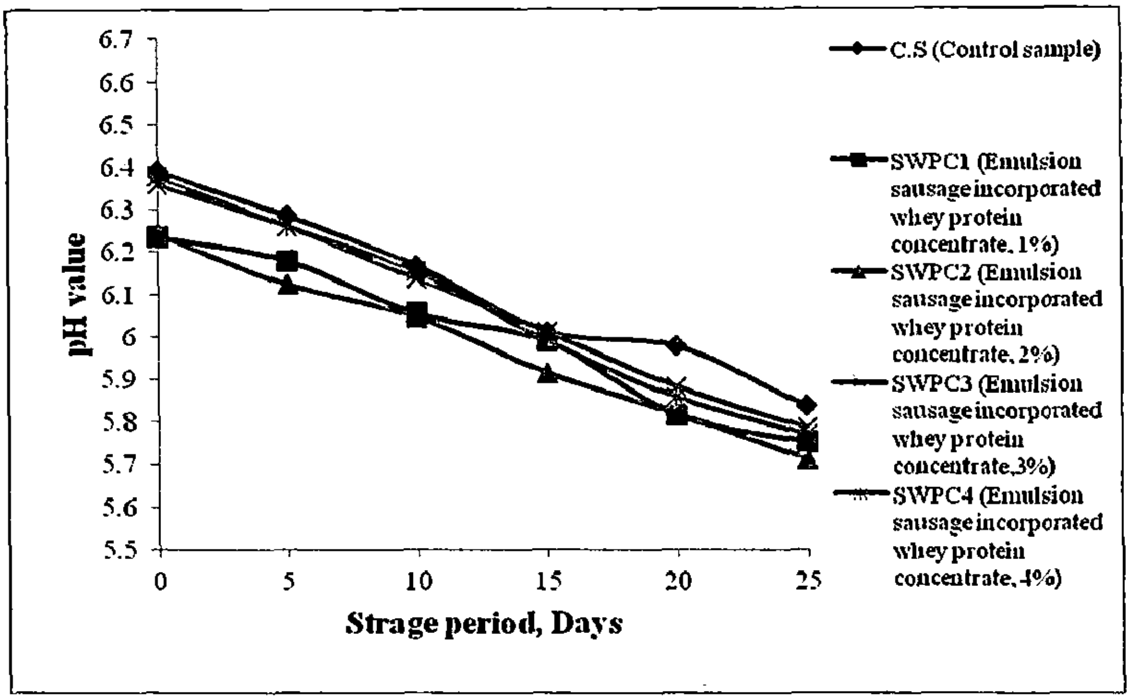



**Figure 4.8:** Regression analysis of pH value of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

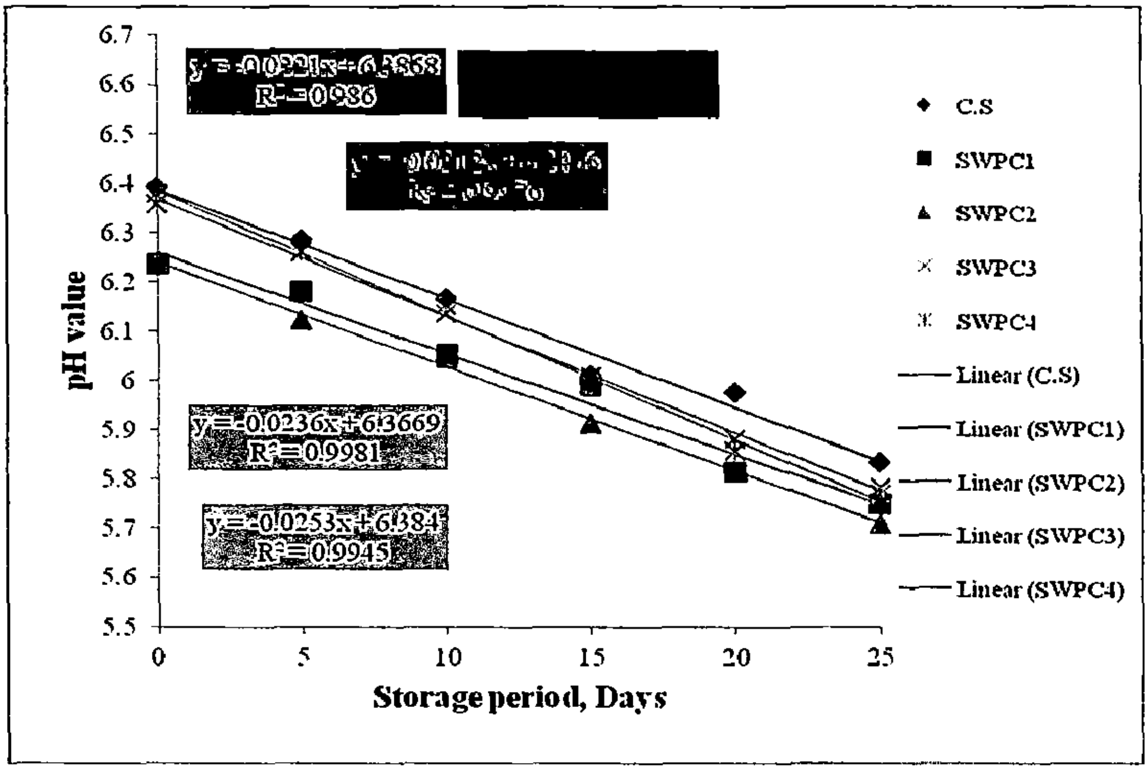


**Table 4.22:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein isolate

| Sample code | pH Value | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 6.390± 0.017ab | 6.286± 0.006 | 6.167± 0.016 | 6.011± 0.048 | 5.976± 0.004 | 5.834± 0.009c |
| $Swpi_1$ | 6.364± 0.097ab | 6.302± 0.030 | 6.262± 0.025 | 6.156± 0.048 | 6.084± 0.061 | 5.902± 0.021d |
| $Swpi_2$ | 6.313± 0.044ab | 6.278± 0.038 | 6.196± 0.020 | 5.998± 0.061 | 5.812± 0.066 | 5.696± 0.024e |
| $Swpi_3$ | 6.292± 0.036b | 6.206± 0.023 | 6.136± 0.020 | 5.986± 0.062 | 5.774± 0.041 | 5.688± 0.014e |
| $Swpi_4$ | 6.288± 0.041b | 6.184± 0.019 | 6.052± 0.054 | 5.902± 0.022 | 5.883± 0.002 | 5.696± 0.018e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.23:** ANOVA of pH value of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 6.956361 | 0.046687 | | |
| Replicate | 4 | 0.010547 | 0.002637 | 1.404232 | 2.46 |
| FA | 4 | 0.66807 | 0.167017 | 88.94679 | 2.46 |
| FB | 5 | 5.852294 | 1.170459 | 623.3394 | 2.3 |
| Comb(A*B) | 29 | 0.235081 | 0.008106 | 4.317056 | 1.63 |
| Error/Res | 107 | 0.200916 | 0.001878 | | |
| LSD | 0.054264 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.9:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein isolate

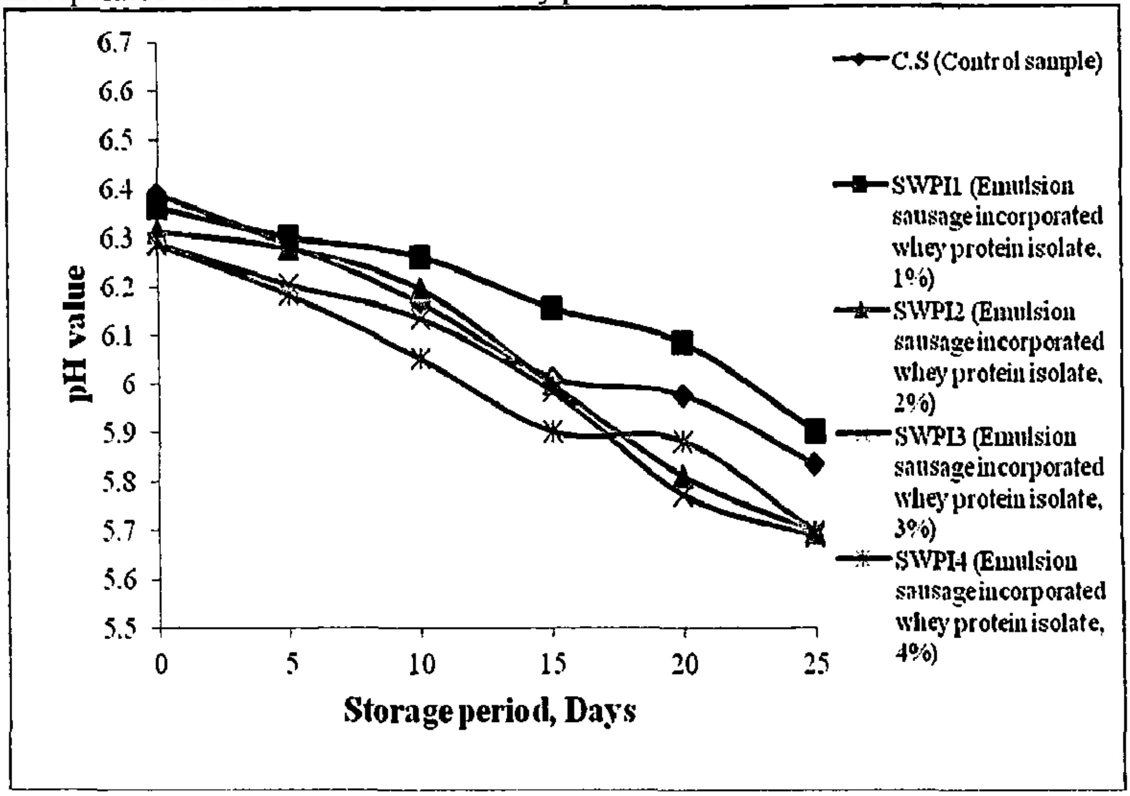



**Fig 4.10:** Regression analysis of pH value of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

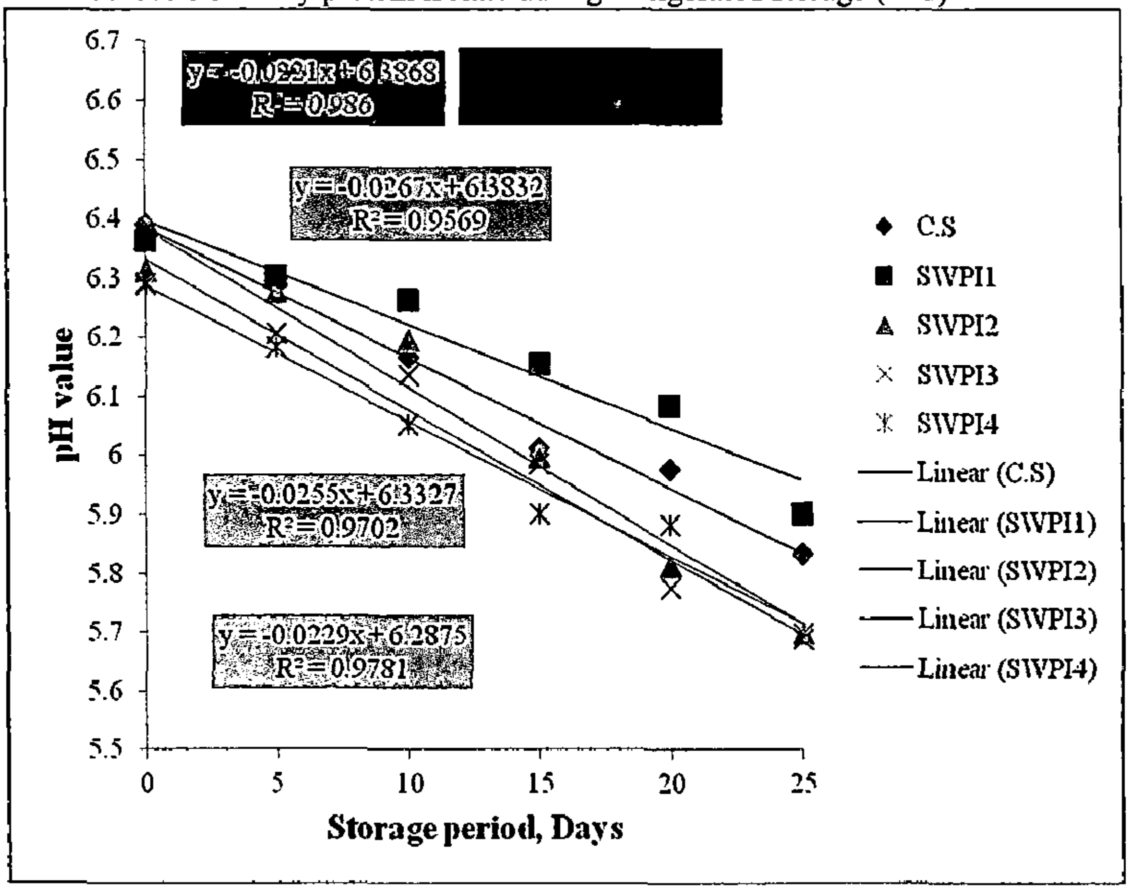


**Table 4.24:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein powder

| Sample code | pH Value | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 6.390± 0.071a | 6.286± 0.006 | 6.167± 0.017 | 6.011± 0.007 | 5.976± 0.004 | 5.834± 0.009c |
| $Swpp_1$ | 6.422± 0.021b | 6.368± 0.005 | 6.286± 0.003 | 6.118± 0.003 | 5.915± 0.005 | 5.760± 0.003d |
| $Swpp_2$ | 6.312± 0.043ac | 6.276± 0.003 | 6.114± 0.002 | 6.076± 0.040 | 5.889± 0.007 | 5.716± 0.004d |
| $Swpp_3$ | 6.350± 0.014ac | 6.234± 0.005 | 6.241± 0.052 | 6.178± 0.005 | 6.013± 0.006 | 5.894± 0.040c |
| $Swpp_4$ | 6.362± 0.017ac | 6.110± 0.004 | 5.992± 0.007 | 5.891± 0.004 | 5.784± 0.002 | 5.668± 0.007e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.25:** ANOVA of pH value of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 7.094154 | 0.047612 | | |
| Replicate | 4 | 0.003937 | 0.000984 | 1.969001 | 2.46 |
| FA | 4 | 0.686012 | 0.171503 | 343.0702 | 2.46 |
| FB | 5 | 6.001163 | 1.200233 | 2400.914 | 2.3 |
| Comb(A*B) | 29 | 0.353489 | 0.012189 | 24.38309 | 1.63 |
| Error/Res | 107 | 0.05349 | 0.0005 | | |
| LSD | 0.027999 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.11:** Effect of refrigerated storage (0°C) on pH value of emulsion sausages incorporated with different levels of whey protein powder

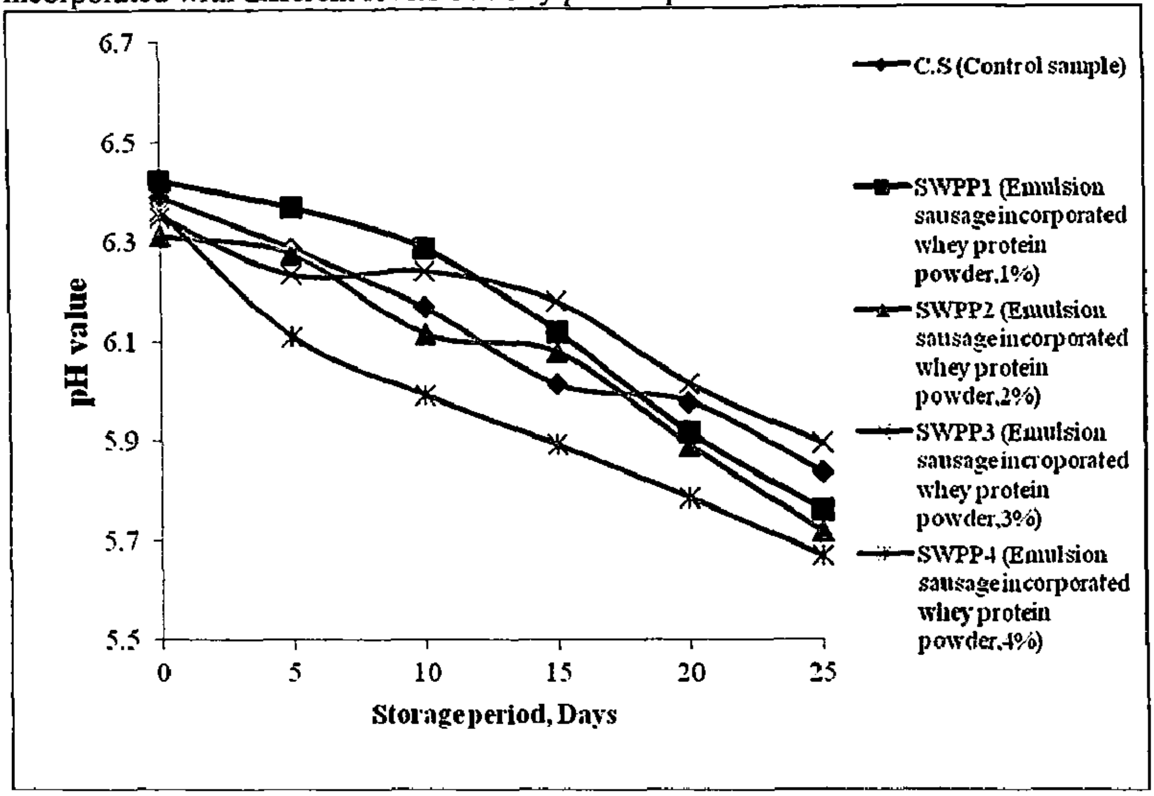
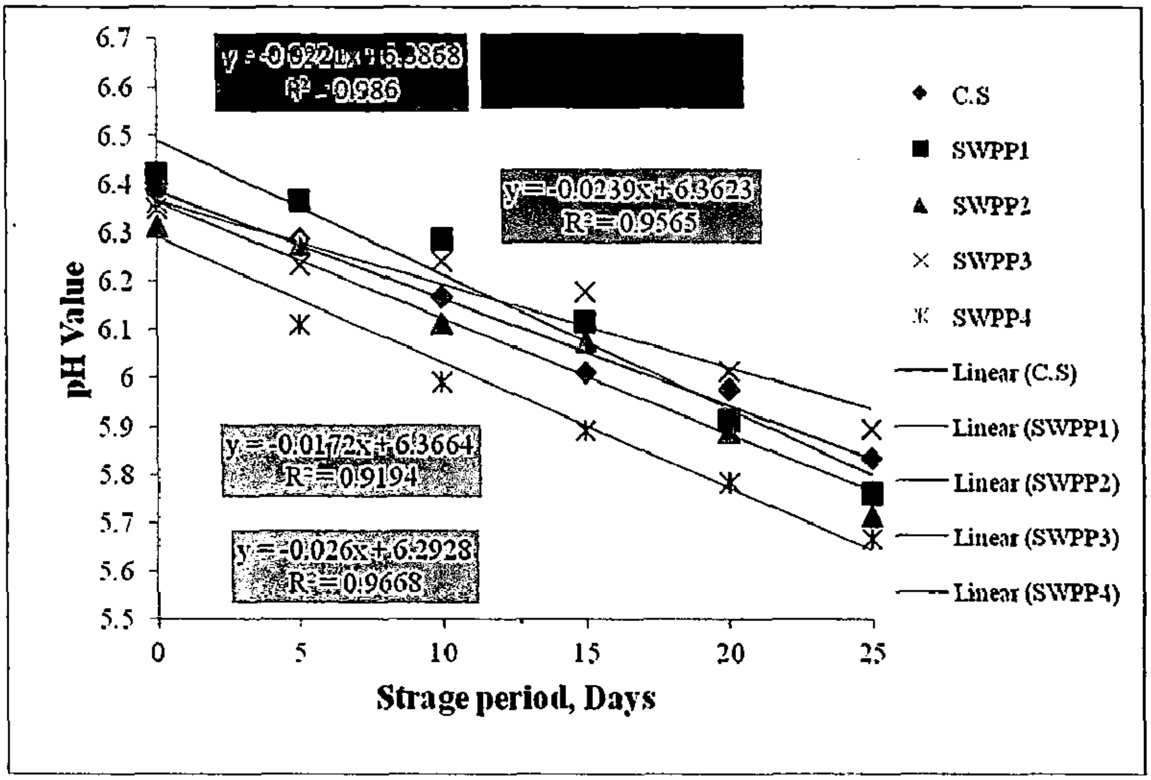



**Fig 4.12:** Regression analysis of pH value of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.2.3 Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.

**a) Emulsion sausage incorporated with whey protein concentrate**

Table 4.26 and Figure 4.13 represent the results of ash content of emulsion sausage incorporated with different levels (1, 2, 3 and 4%) of whey protein concentrate. In fresh condition, ash contents of all samples were found in the range of 2.11- 2.17%. During refrigerated storage at 0°C ash content increased slightly and it was found in the range of 2.48-2.61% at the end of 25th day of storage. Different levels of whey protein concentrate, refrigerated storage and their interaction significantly ($p<0.05$) increased ash content of emulsion sausages (ANOVA Table 4.27).

The equation of regression lines and correlation coefficient of all samples with different levels (1, 2, 3 and 4%) of whey protein concentrate have been shown on the regression graph (Fig 4.14). The positive sign in the coefficients of x explains that there was constant increase of ash content during storage. The values of $R^2$ for all samples at different levels (1, 2, 3 and 4%) of whey protein concentrate were between 0.9231 – 09968. The values of $R^2$ were near to 1, therefore the graph may be approximated to a straight line and linear relation well fits between storage period and pH values.

**b) Emulsion sausage incorporated with whey protein isolate**

The ash contents of all samples of emulsion sausages incorporated with whey protein isolate at the level (1, 2, 3 and 4%) were found in the range of 2.12-2.29% just after preparation. Samples incorporated with whey isolate had significantly ($p<0.05$) lower percentage of ash content compared to the control sample. The results of ash content of emulsion sausages have been presented in Table 4.28. Ash content of emulsion sausage is evaluated before and during refrigerated storage at 0°C. The ANOVA (Table 4.29) results indicate that refrigerated storage significantly ($p<0.05$) increased the ash content of emulsion sausages. At the end of 25th day of storage, ash content of emulsion sausage was found in the range of 2.44-2.62%. Fig 4.15 shows the protein content profile of emulsion sausages samples during refrigerated storage (0°C).

The equation of regression lines and correlation coefficient of all samples of emulsion sausages incorporated with whey protein isolate with different levels 1, 2, 3 and 4% have been shown on the regression graph (Figure 4.16). The positive sign in the coefficients of x explained that there was slight increase of ash content during refrigerated storage. The correlation coefficient value explains the profile of ash content during storage period in days. The values of $R^2$ were between 0.966-0.9963. The increasing nature of ash content with storage time was perfect very near to 1,and it indicated that correlation are almost perfect and the graphs may be approximated to a straight line.

**c) Emulsion sausage incorporated with whey protein powder**

Table 4.30 presents the results of ash content of buffalo meat emulsion sausages prepared with different levels (1, 2, 3 and 4%) of whey protein powder during refrigerated storage at 0° C. The ash content of all samples of sausages were found in the range of 2.18- 2.54% in fresh condition. A higher ash content as compared to raw meat was possibly resulted from salt and other additives added viz whey protein powder (Visessanguan *et al.*, 2005). According to Meltem (2006), addition of whey protein in raw meatballs increased slightly the ash content, and four percent WP almost doubled the ash content when compared with that of the control. During refrigerated storage there was a significant ($p<0.05$) increase of ash content of emulsion sausage samples. That might be due to loss of moisture content during storage. The ash content of control sample was found to be 2.72% for emulsion sausage treated with different levels (1, 2, 3 and 4%) of whey protein powder had ash contents in the range 2.37-2.67% on 25th day of storage. The result of ANOVA (Table 4.25) showed that treatment of whey protein powder found a significantly ($p<0.05$) affected ash content of emulsion sausage samples. Fig 4.17 indicated that the ash content of emulsion sausages samples was found to increase during refrigerated storage at 0°C.

The equation of regression lines and correlation coefficient of all samples with different levels (1, 2, 3 and 4%) of whey protein powder have been shown on the regression graph (Fig 4.18). The positive sign in the coefficients of x explains that there was constant increase of ash content during storage. The values of $R^2$ for all samples at levels of 1, 2, 3 and 4% whey protein powder were in the range of 0.9903-0.9954. The values of $R^2$ were very near to 1, thus the graph may be approximately exhibited by linear system and this linear relation well fits between storage periods and ash content values.

**Table 4.26:** Effect of refrigerated storage (0°C) on ash content of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample code | Ash Content% | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 2.36± 0.027a | 2.48± 0.022 | 2.52± 0.011 | 2.57± 0.020 | 2.64± 0.008 | 2.72± 0.013f |
| Swpc$_1$ | 2.11± 0.021b | 2.17± 0.013 | 2.22± 0.011 | 2.33± 0.027 | 2.40± 0.014 | 2.48± 0.014g |
| Swpc$_2$ | 2.13± 0.028c | 2.20± 0.019 | 2.27± 0.019 | 2.37± 0.008 | 2.45± 0.015 | 2.54± 0.016h |
| Swpc$_3$ | 2.15± 0.022d | 2.19± 0.011 | 2.23± 0.024 | 2.34± 0.025 | 2.46± 0.017 | 2.58± 0.008i |
| Swpc$_4$ | 2.17± 0.030e | 2.25± 0.015 | 2.26± 0.022 | 2.34± 0.066 | 2.46± 0.013 | 2.61± 0.015j |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs= Control sample, Swpc$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.27:** ANOVA of ash content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 4.258733 | 0.028582 | | |
| Replicate | 4 | 0.00448 | 0.00112 | 2.008043 | 2.46 |
| FA | 4 | 1.284213 | 0.321053 | 575.6151 | 2.46 |
| FB | 5 | 2.842765 | 0.568553 | 1019.356 | 2.3 |
| Comb(A*B) | 29 | 0.072075 | 0.002485 | 4.455943 | 1.63 |
| Error/Res | 107 | 0.05968 | 0.000558 | | |
| LSD | 0.029575 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.13:** Effect of refrigerated storage (0°C) on ash content of emulsion sausages incorporated with different levels of whey protein concentrate

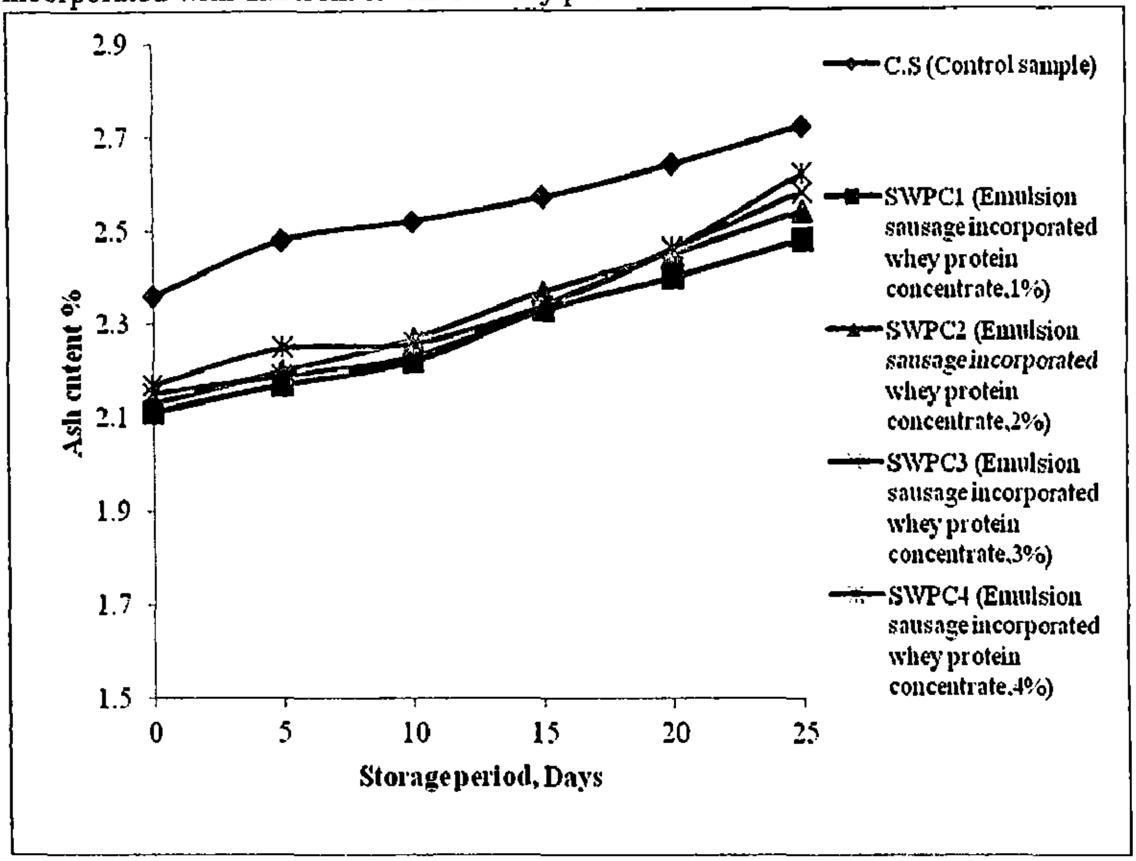



**Fig 4.14:** Regression analysis of ash content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

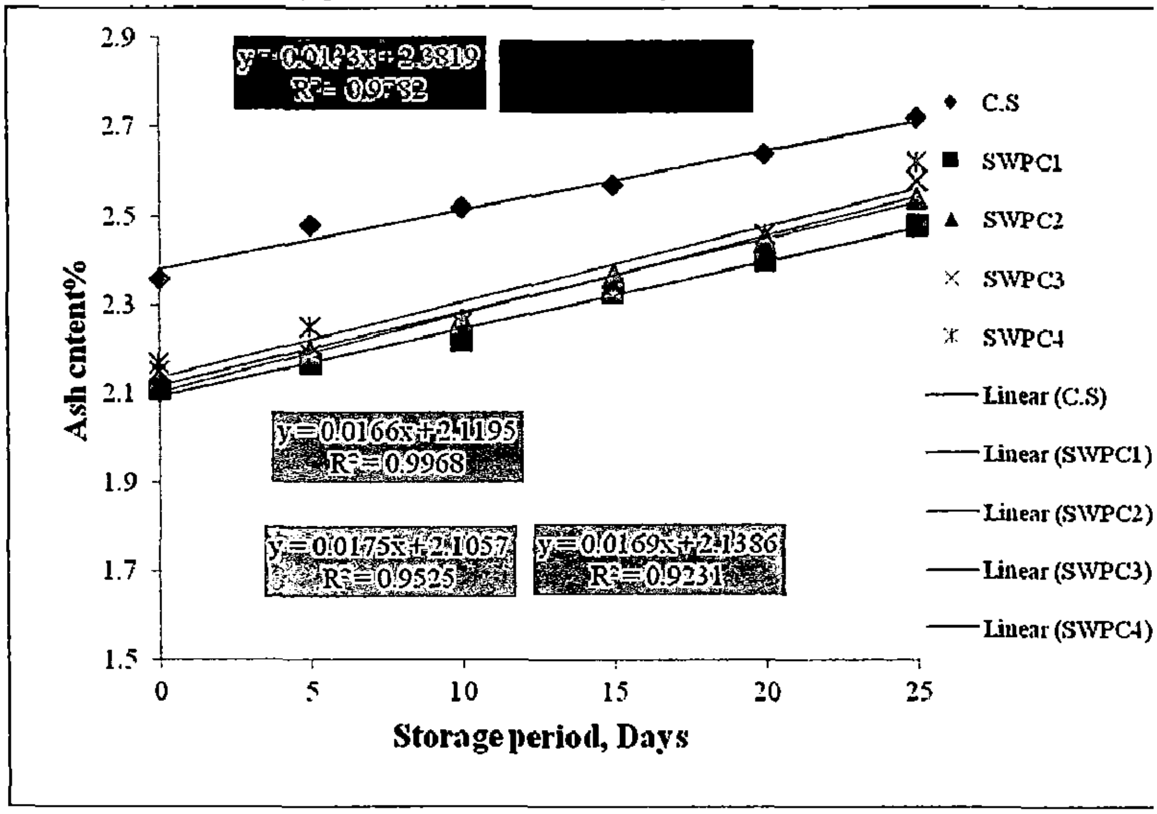


**Table 4.28:** Effect of refrigerated storage (0°C) on ash content of emulsion sausages incorporated with different levels of whey protein isolate

| Control Sample | Ash Content% | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period, Days | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 2.36± 0.027a | 2.48± 0.022 | 2.52± 0.011 | 2.57± 0.020 | 2.64± 0.008 | 2.72± 0.013e |
| **Swpi$_1$** | 2.29± 0.055b | 2.32± 0.011 | 2.37± 0.016 | 2.43± 0.015 | 2.52± 0.011 | 2.61± 0.017f |
| **Swpi$_2$** | 2.25± 0.030c | 2.31± 0.015 | 2.37± 0.020 | 2.46± 0.011 | 2.54± 0.011 | 2.60± 0.022f |
| **Swpi$_3$** | 2.24± 0.008c | 2.27± 0.015 | 2.34± 0.011 | 2.44± 0.019 | 2.55± 0.016 | 2.62± 0.011f |
| **Swpi$_4$** | 2.12± 0.027d | 2.17± 0.011 | 2.23± 0.011 | 2.30± 0.008 | 2.37± 0.013 | 2.44± 0.015g |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpi$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.29:** ANOVA of ash content of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 3.262429 | 0.021895 | | |
| **Replicate** | 4 | 0.003769 | 0.000942 | 2.218963 | 2.46 |
| **FA** | 4 | 1.024416 | 0.256104 | 603.0618 | 2.46 |
| **FB** | 5 | 2.098837 | 0.419767 | 988.4489 | 2.3 |
| **Comb(A*B)** | 29 | 0.093736 | 0.003232 | 7.611213 | 1.63 |
| **Error/Res** | 107 | 0.04544 | 0.000425 | | |
| **LSD** | 0.025806 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.15:** Effect of refrigerated storage (0°C) on ash content of emulsion sausages incorporated with different levels of whey protein isolate

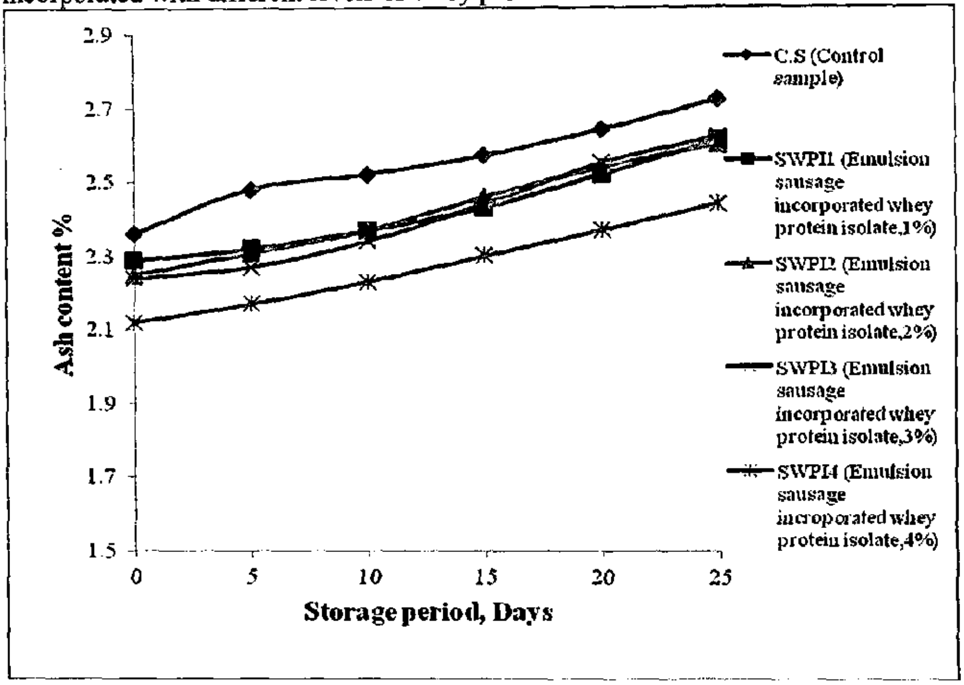



**Fig 4.16:** Regression analysis of ash content of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage at 0°C

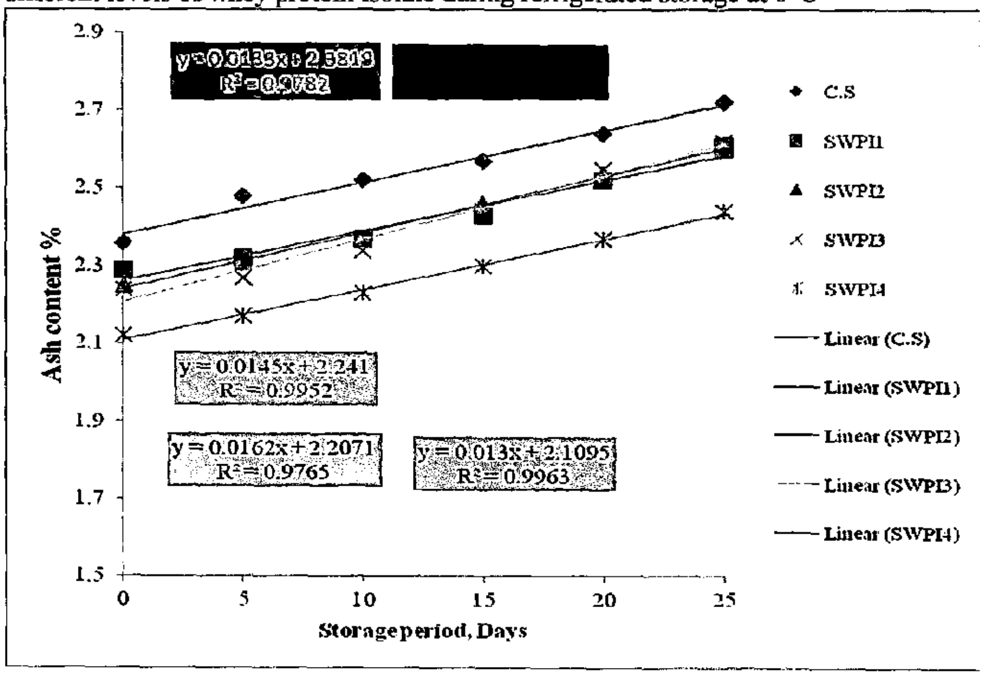


**Table 4.30:** Effect of refrigerated storage (0°C) on ash content of emulsion sausages incorporated with different levels of whey protein powder

| Control Sample | Ash Content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period, Days | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 2.36± 0.027a | 2.48± 0.020 | 2.52± 0.011 | 2.57± 0.020 | 2.65± 0.008 | 2.72± 0.020e |
| **Swpp$_1$** | 2.36± 0.056a | 2.40± 0.011 | 2.46± 0.025 | 2.51± 0.008 | 2.57± 0.013 | 2.51± 0.008f |
| **Swpp$_2$** | 2.18± 0.032b | 2.22± 0.011 | 2.30± 0.008 | 2.37± 0.008 | 2.44± 0.016 | 2.37± 0.008a |
| **Swpp$_3$** | 2.54± 0.074c | 2.59± 0.015 | 2.62± 0.001 | 2.67± 0.013 | 2.72± 0.014 | 2.67± 0.013g |
| **Swpp$_4$** | 2.43± 0.045d | 2.52± 0.015 | 2.58± 0.008 | 2.63± 0.008 | 2.68± 0.008 | 2.63± 0.008h |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpp$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.31:** ANOVA of ash content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 3.432899 | 0.02304 | | |
| **Replicate** | 4 | 0.003449 | 0.000862 | 1.287963 | 2.46 |
| **FA** | 4 | 1.690043 | 0.422511 | 631.0531 | 2.46 |
| **FB** | 5 | 1.621275 | 0.324255 | 484.3006 | 2.3 |
| **Comb(A*B)** | 29 | 0.049941 | 0.001722 | 2.572115 | 1.63 |
| **Error/Res** | 107 | 0.07164 | 0.00067 | | |
| **LSD** | 0.032403 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.17:** Effect of refrigerated storage (0°C) on ash content of **emulsion** sausages incorporated with different levels of whey protein powder

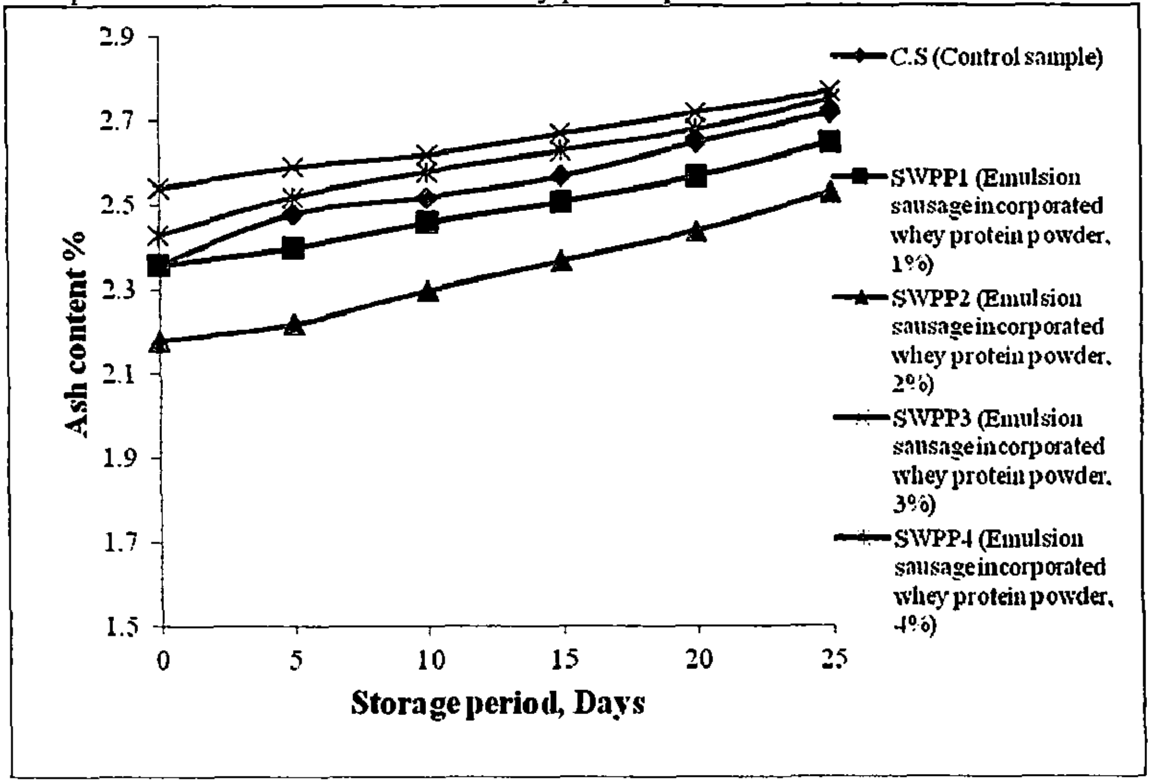
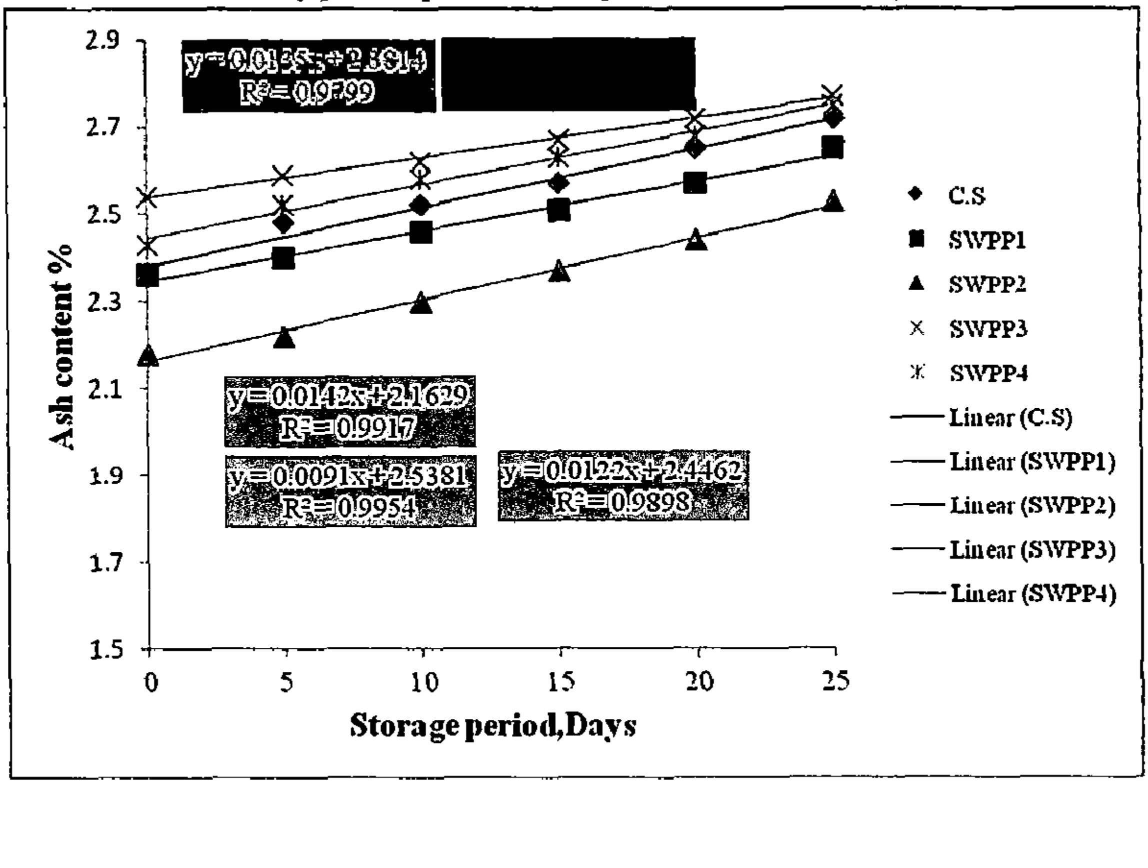



**Fig 4.18:** Regression analysis of ash content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.2.4 Protein content

Protein content is indicative of nutritional quality. In case of meat products, the quality of protein is very high, because of the presence of most of the essential amino acids. Protein was found to also degrade slowly by micro organisms during refrigerated storage.

**a) Emulsion sausage incorporated with protein concentrate**

Protein contents of all samples of emulsion sausages have been given in Table 4.32. In fresh condition the protein contents of all samples of emulsion sausages incorporated with whey protein concentrate with different levels (1, 2, 3 and 4%) were found to be 23.55, 23.96, 24.06 and 24.10% respectively. Samples incorporated with whey concentrate have significantly ($p<0.05$) higher percentage of protein content. Refrigerated storage significantly ($p<0.05$) increased the protein content of emulsion sausages. Hence at the end of $25^{th}$ day of storage, protein content of emulsion sausage treated with the different levels of whey protein concentrate 1, 2, 3 and 4% were found to be 26.43, 26.94, 27.06 and 27.13% respectively (Figure 4.19).

The equation of regression lines and correlation coefficients of all samples of emulsion sausages (control and treated with whey protein concentrate) have been shown on the regression graph (Figure 4.20). The positive sign in the coefficients of x explains that there was constant increase of protein content during storage period. The values of $R^2$ were in the range of 0.9843-0.9976. It was nearly to 1, thus the graph may be approximated to a straight line and linear relation well fits between storage period and protein content values.

**b) Emulsion sausage incorporated with protein isolate**

The protein contents of all samples of emulsion sausages (control and treated with whey protein isolate at different levels 1, 2, 3 and 4%) were found in the range of 23.38% - 24.42% just after preparation (Table 4.34). Samples treated with whey protein isolate had significantly ($p<0.05$) higher percentage of protein content as compared to control sample (22.58%). Refrigerated storage significantly ($p<0.05$) increased the protein content of emulsion sausages. Therefore the ends of $25^{th}$ day of storage protein content of emulsion sausage were found in the range of 26.48 to 27.11%. Fig 4.21 shows the protein content profile of emulsion sausages samples during refrigerated storage (0°C). Different levels of WPI significantly ($p<0.05$) affected the protein content of ES (ANOVA Table 4.35).

The equation of regression lines and correlation coefficient of all samples of emulsion sausages incorporated with whey protein isolate with different levels (1, 2, 3 and 4%) have been shown on the regression graph (Figure 4.22). The positive sign in the coefficients of x explains that there was slight increase of protein content during refrigerated storage. The correlation coefficient values explain the profile of protein content with storage time in days. The values of $R^2$ were found between 0.9843-0.9979. It explained linear behavior of protein increased during storage.

**c) Emulsion sausage incorporated with protein powder**

Different levels of whey protein powder significantly ($p<0.05$) affected the protein content of sausage samples. The protein contents of treated emulsion sausages with different levels (1, 2, 3 and 4%) were found in the range of 24.90-25.22% in fresh condition (Table 4.36). Meltem and Eylem (2004) stated emulsion type Turkey roll incorporated with whey protein had lower protein content than control. This lower value may due to lower quantity of protein (13%) in WP. In another study, Meltem (2006) reported that whey protein did not affect protein contents of the raw and cooked meat balls. Figure 4.23 depicts the protein content profile of emulsion sausage (control and treated) during refrigerate storage (0°C). The protein content during storage was found to increase consistently. ANOVA (Table 4.37) results indicated the refrigerated storage significantly ($p<0.05$) increased the protein content of emulsion sausage. Perhaps this happened due to the loss of moisture content during the storage period. At the end of $25^{th}$ day, of storage protein contents of sausage samples were found in the range of 27.45-27.92%. Bhaskar *et al.*, (2009), advocated that the protein content of pork sausages incorporated with low, medium and high calcium milk had caused a significant ($p<0.05$) increase at refrigerated storage (7±1°C), This might be due to the loss of moisture content. Similar results of increasing crude protein were also obtained by baker *et al.*, (1970) and Reddy and Vijaylakshmi (1998) in chicken meat sausages.

The positive sign in the coefficient of x explains that there was a constant increase of protein content during storage period (Figure 4.24). The values of $R^2$ were between 0.9889-0.9979. The values of $R^2$ were near to 1 thus the graph may be approximated to a straight line and linear system well fits between storage period and protein content values.

**Table 4.32:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample code | Protein Content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 22.58± 0.052a | 23.35± 0.020 | 23.95± 0.023 | 24.56± 0.051 | 25.12± 0.039 | 25.82± 0.034e |
| **Swpc$_1$** | 23.55± 0.016a | 24.02± 0.040 | 24.83± 0.054 | 25.36± 0.038 | 25.91± 0.066 | 26.43± 0.038f |
| **Swpc$_2$** | 23.96± 0.018b | 24.46± 0.040 | 25.22± 0.036 | 25.85± 0.036 | 26.34± 0.035 | 26.94± 0.043g |
| **Swpc$_3$** | 24.06± 0.023c | 24.88± 0.032 | 25.49± 0.027 | 25.91± 0.054 | 26.41± 0.025 | 27.06± 0.048h |
| **Swpc$_4$** | 24.10± 0.013d | 24.93± 0.047 | 25.65± 0.053 | 26.06± 0.043 | 26.51± 0.047 | 27.13± 0.024i |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpc$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.33:** ANOVA of protein content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 201.6655 | 1.35346 | | |
| **Replicate** | 4 | 0.022843 | 0.005711 | 3.16733 | 2.46 |
| **FA** | 4 | 45.35095 | 11.33774 | 6288.295 | 2.46 |
| **FB** | 5 | 155.4032 | 31.08063 | 17238.38 | 2.3 |
| **Comb(A*B)** | 29 | 0.718515 | 0.024776 | 13.74182 | 1.63 |
| **Error/Res** | 107 | 0.19292 | 0.001803 | | |
| **LSD** | 0.053173 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.19:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein concentrate

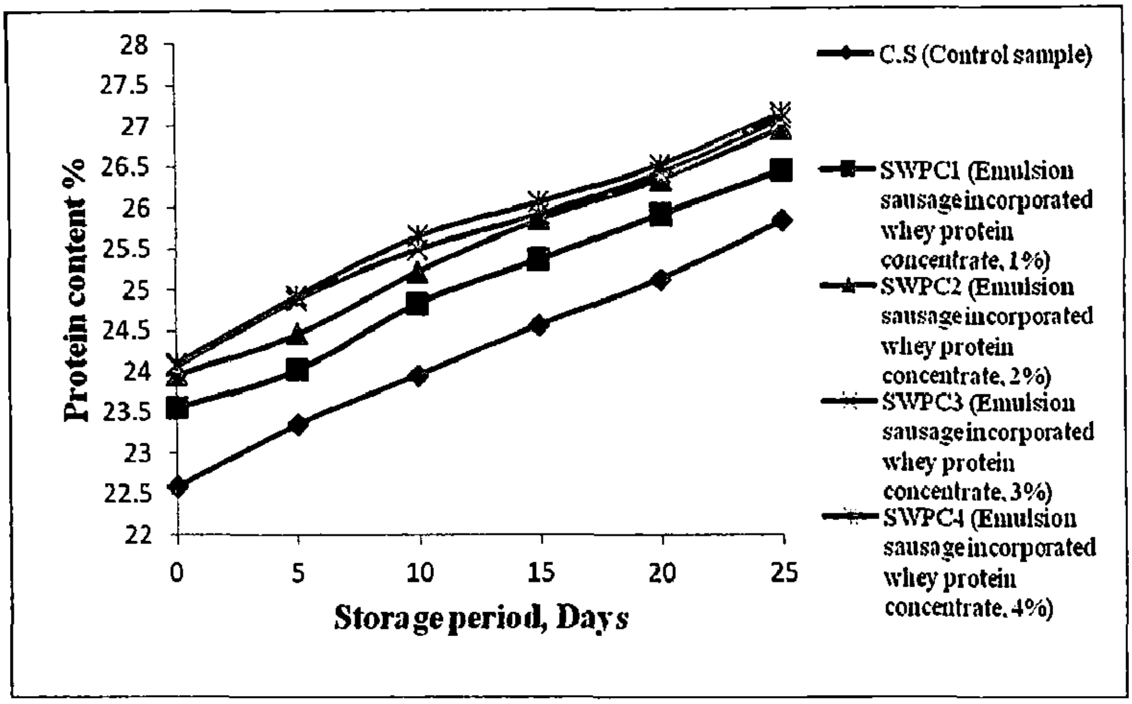



**Fig 4.20:** Regression analysis of protein content of emulsion sausages incorporated with different levels of whey protein concentrate refrigerated during storage (0°C)

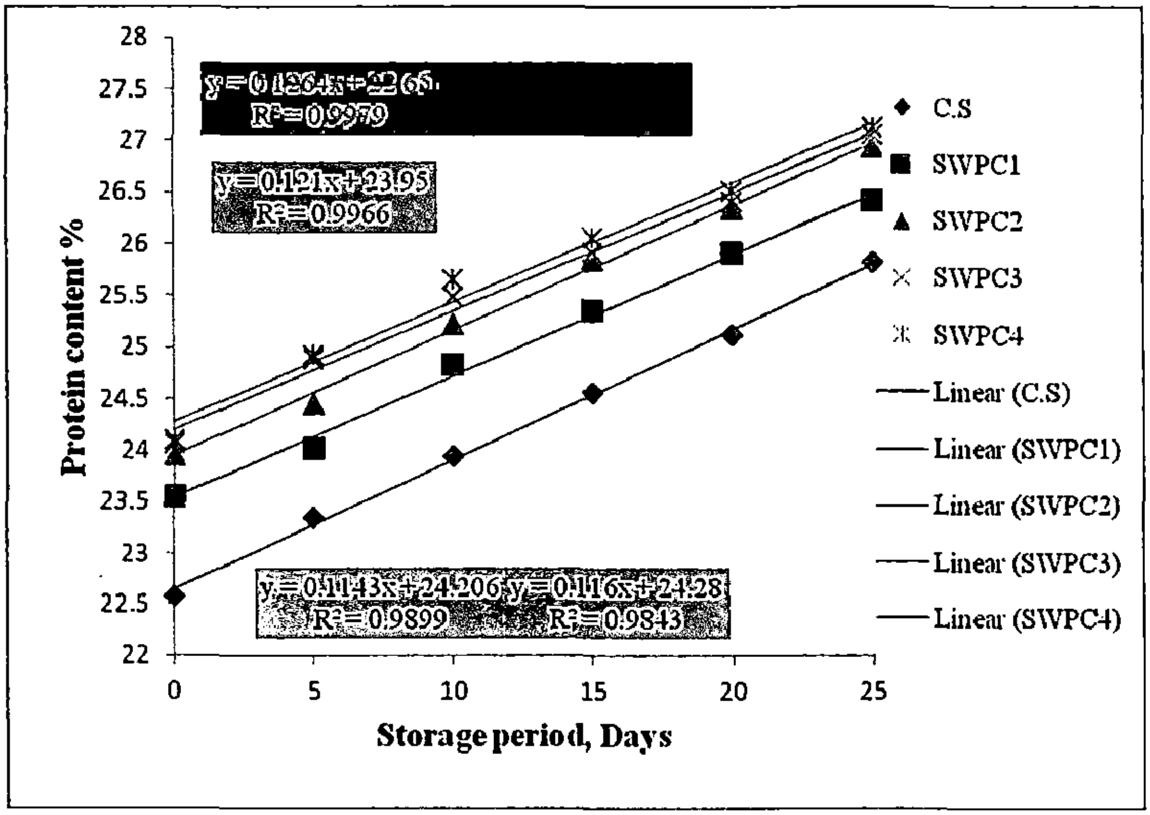


**Table 4.34:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein isolate

| Sample code | Protein Content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **C.S** | 22.58± 0.052a | 23.35± 0.020 | 23.95± 0.023 | 24.56± 0.051 | 25.12± 0.039 | 25.82± 0.034f |
| **$Swpi_1$** | 23.38± 0.046b | 23.91± 0.043 | 24.48± 0.052 | 25.12± 0.096 | 25.76± 0.039 | 26.82± 0.046g |
| **$Swpi_2$** | 23.84± 0.023c | 24.26± 0.44 | 24.91± 0.071 | 25.44± 0.059 | 26.20± 0.054 | 26.48± 0.061h |
| **$Swpi_3$** | 24.27± 0.023d | 24.87± 0.056 | 25.32± 0.039 | 25.88± 0.045 | 26.44± 0.049 | 27.11± 0.069i |
| **$Swpi_4$** | 24.42± 0.023e | 24.10± 0.061 | 24.65± 0.051 | 25.23± 0.039 | 25.92± 0.041 | 26.79± 0.094j |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.35:** ANOVA of protein content of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 188.5121 | 1.265182 | | |
| **Replicate** | 4 | 0.008209 | 0.002052 | 0.713079 | 2.46 |
| **FA** | 4 | 40.74193 | 10.18548 | 3538.923 | 2.46 |
| **FB** | 5 | 145.7714 | 29.15429 | 10129.59 | 2.3 |
| **Comb(A*B)** | 29 | 1.690831 | 0.058305 | 20.25777 | 1.63 |
| **Error/Res** | 107 | 0.30796 | 0.002878 | | |
| **LSD** | 0.067182 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.21:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein isolate

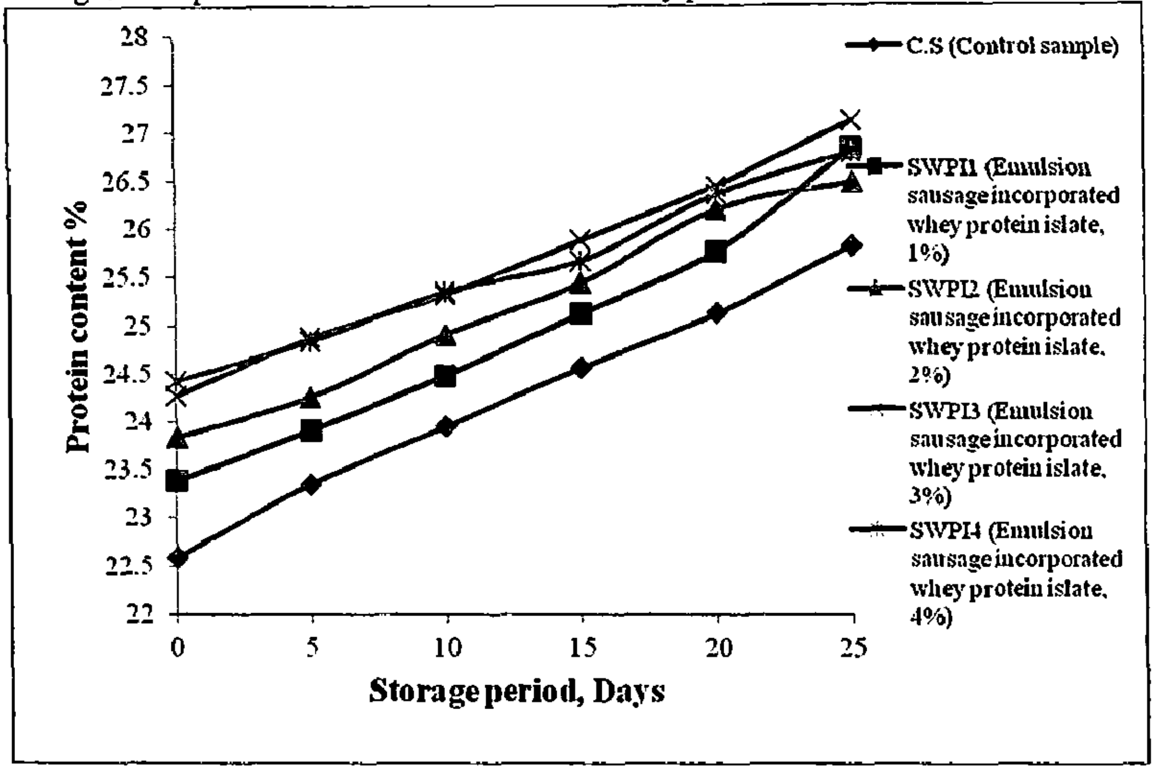



**Fig 4.22:** Regression analysis of protein content of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

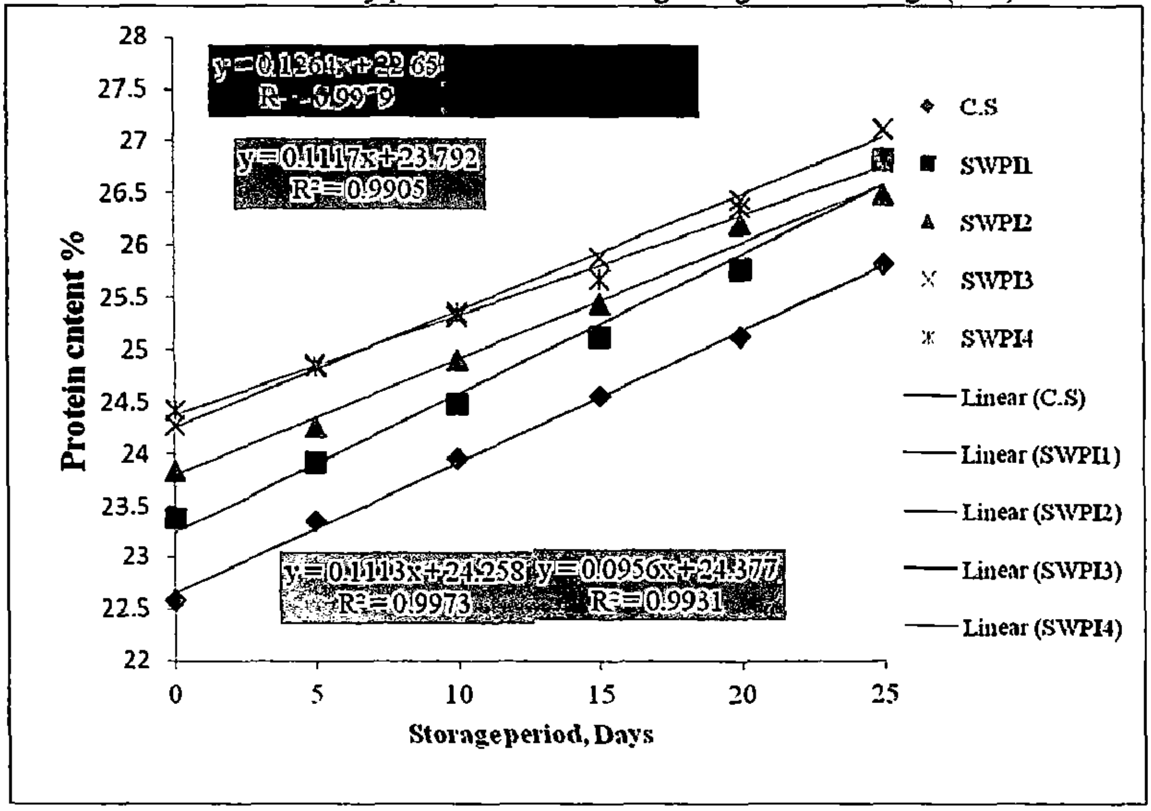


**Table 4.36:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein powder

| Sample code | Protein content% | | | | | |
|---|---|---|---|---|---|---|
| | Storage Periods, Days | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| C.S | 22.58± 0.052a | 23.35± 0.020 | 23.95± 0.023 | 24.56± 0.051 | 25.12± 0.039 | 25.82± 0.034e |
| $Swpp_1$ | 24.90± 0.032b | 25.35± 0.033 | 25.92± 0.060 | 26.44± 0.036 | 26.93± 0.038 | 27.78± 0.048f |
| $Swpp_2$ | 24.96± 0.096b | 25.41± 0.019 | 26.07± 0.040 | 26.35± 0.046 | 27.13± 0.031 | 27.45± 0.051g |
| $Swpp_3$ | 25.12± 0.048c | 25.76± 0.036 | 26.18± 0.040 | 26.67± 0.064 | 27.22± 0.041 | 27.92± 0.055h |
| $Swpp_4$ | 25.22± 0.041d | 25.84± 0.023 | 26.32± 0.051 | 26.75± 0.039 | 27.31± 0.034 | 27.81± 0.032i |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.37:** ANOVA of protein content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 248.0114 | 1.664506 | | |
| **Replicate** | 4 | 0.007411 | 0.001853 | 0.951043 | 2.46 |
| **FA** | 4 | 113.1827 | 28.29566 | 14525.22 | 2.46 |
| **FB** | 5 | 133.1329 | 26.62659 | 13668.42 | 2.3 |
| **Comb(A*B)** | 29 | 1.487397 | 0.05129 | 26.32884 | 1.63 |
| **Error/Res** | 107 | 0.20844 | 0.001948 | | |
| **LSD** | 0.055271 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Figure 4.23:** Effect of refrigerated storage (0°C) on protein content of emulsion sausages incorporated with different levels of whey protein powder

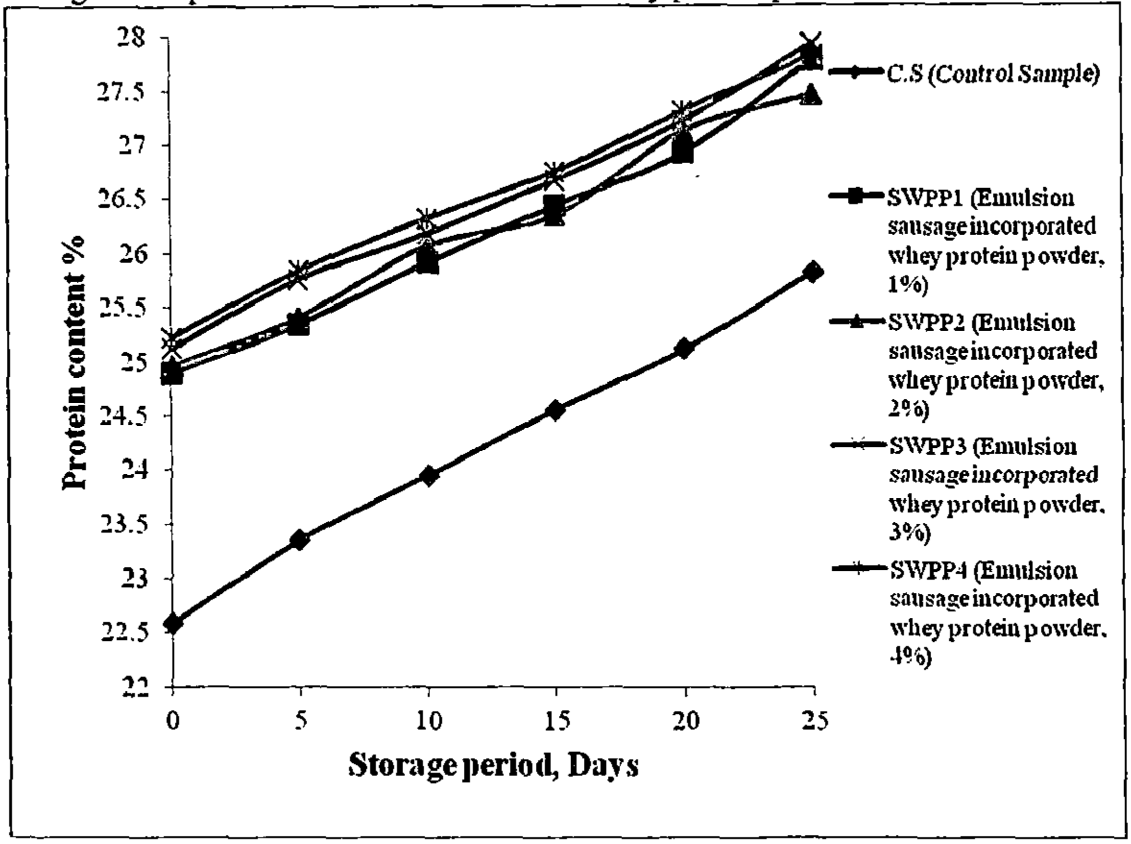
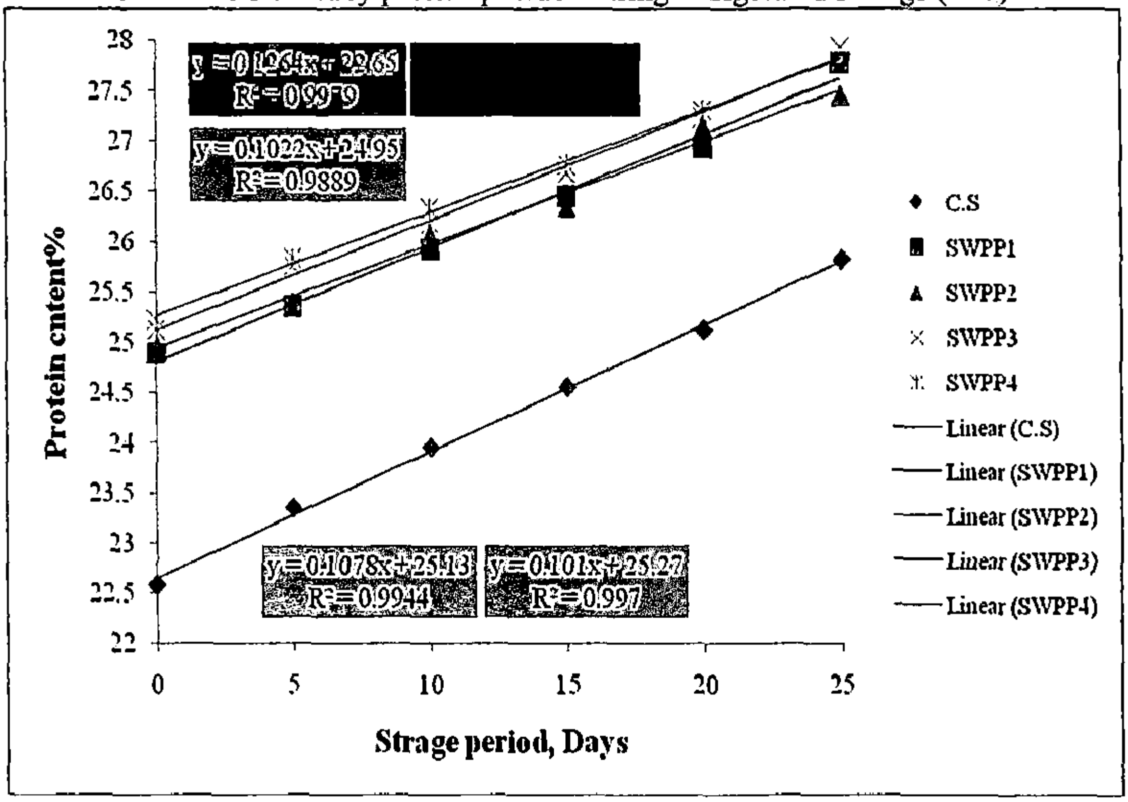



**Fig 4.24:** Regression analysis of protein content of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



### 4.2 .2.5 Fat content

**a) Emulsion sausage incorporated with protein concentrate**

Table 4.38 and Figure 4.25 represent the results of fat content of emulsion sausage incorporated with whey protein concentrate with levels of 1, 2, 3 and 4%. Fat content values were between 12.69-12.95% in fresh condition. So control sample had significantly ($p<0.05$) higher fat content percentage as compared to the emulsion sausages incorporated with whey protein concentrate. During refrigerated storage (0°C), the fat contents of sausage samples (control and treated) were found to increase. The fat content after 25 days was found in the range of 14.61-15.44%. Different levels of whey protein concentrate significantly ($p<0.05$) decreased fat content of emulsion sausages. Dharmaveer *et al.*, (2007) reported that fat content of stored sausages was significantly ($p<0.01$) increased during the storage. All the levels of WPC significantly ($p<0.05$) affected the fat content of emulsion sausage sample (ANOVA Table 4.39).

The equation of regression lines and correlation coefficient of all samples of emulsion sausages incorporated with whey protein concentrate with the different levels (1, 2, 3, and 4%) has been shown on the regression graph (Figure 4.26). The positive sign in the coefficients of x explain that there was constant increase of protein content during storage period. Therefore the values of $R^2$ were between 0.9703 - 0.9912 and the values of $R^2$ were near to 1, thus the graph may be approximated to a straight line and linear relation well fits between drying period and protein content values.

**b) Emulsion sausage incorporated with protein isolate**

The fat contents of all samples of emulsion sausages incorporated with different levels of (1, 2, 3 and 4%) whey protein isolate were found in the range of 11.21% to 11.99% in fresh condition. Samples incorporated with whey isolate have significantly ($p<0.05$) lower percentage of fat content as compared to control sample (13.47%). The results of fat estimation have been presented in Table 4.40. Fat content of emulsion sausage was evaluated before and during refrigerated storage at 0°C. Refrigerated storage significantly ($p<0.05$) increased the fat content of emulsion sausages. Accordingly at the end of 25$^{th}$ day of storage fat content of emulsion sausage was found in the range of 14.12 to 14.69%. Fig 4.27 shows the protein content profile of emulsion sausages samples during refrigerated storage (0°C). The (ANOVA Table 4.41) showed that there was a significant ($p<0.05$) affect on fat content of emulsion sausage samples treated with different levels of whey protein powder.

The equation of regression lines and correlation coefficient of all samples of emulsion sausages incorporated with different levels (1, 2, 3 and 4%) whey protein isolate have been shown on the regression graph (Figure 4.28). The positive sign in the coefficients of x explains that there was a slight increase of fat content during refrigerated storage. The correlation coefficient values explained that linear relation existed between fat content and storage period, days. The values of $R^2$ were between 0.9812-0.9968. The increasing nature of fat content with storage time was perfect at $R^2=1$, and the values of $R^2$ for all samples were found near 1 which shows that correlation were almost perfect and the graphs may be approximated to a straight line.

**c) Emulsion sausage incorporated with protein powder**

Fat contents of fresh emulsion sausage incorporated with different levels (1, 2, 3 and 4%) whey protein powder were found between 11.95-12.23 % (Table 4.42). During refrigerated storage fat contents of all the samples was found to a significantly ($p<0.05$) increase. That might be due to loss of moisture during storage. At the end of 25th day of storage, fat contents were found in the range of 14.67-15.10% (Figure 4.29). According to Soyer and Ertas (2007) the fat content of the *Sucuk* samples significantly ($p<0.05$) affected the moisture, protein and fat contents. Meltem (2006) explained that whey protein did not affect fat contents of the raw and cooked meat balls. Similar results found by Serdaroğlu and Özsümer (2003), these study revealed that incorporation of whey powder did not affect the fat content of cooked beef sausage. But the present study concluded that addition of whey protein powder in emulsion sausages caused a slight decrease of fat content of sausage samples. However, the ANOVA showed that these results statistically significant ($p<0.05$) for fat content of emulsion sausages incorporated with whey protein products (Table 4.43).

The equation of regression lines and correlation coefficient of all samples including control and samples with four levels of whey protein powder have been shown on the regression graph (Fig 4.30). The positive sign in the coefficients of x explains that there was constant increase of fat content during storage. The values of $R^2$ for all samples with different levels (1, 2, 3 and 4%) whey protein powder were in between 0.9597-0.9943. The values of $R^2$ were very near to 1, thus the graph may be approximated to a straight line and linear relation well fits between storage period and fat content values.

**Table 4.38:** Effect of refrigerated storage (0°C) on fat content of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Fat Content % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 13.47± 0.015a | 13.88± 0.025 | 14.14± 0.015 | 14.81± 0.011 | 15.46± 0.021 | 15.73± 0.019f |
| $Swpc_1$ | 12.95± 0.021b | 13.29± 0.013 | 13.85± 0.015 | 14.12± 0.015 | 14.94± 0.024 | 15.20± 0.016g |
| $Swpc_2$ | 12.84± 0.020c | 13.19± 0.011 | 13.70± 0.013 | 14.20± 0.016 | 14.89± 0.019 | 15.44± 0.013h |
| $Swpc_3$ | 12.73± 0.024d | 12.84± 0.018 | 13.23± 0.041 | 13.82± 0.016 | 14.34± 0.022 | 14.61± 0.015i |
| $Swpc_4$ | 12.69± 0.013e | 12.82± 0.029 | 13.41± 0.011 | 13.91± 0.015 | 14.64± 0.026 | 14.81± 0.001j |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.39:** ANOVA of fat content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 119.7536 | 0.803716 | | |
| Replicate | 4 | 0.000219 | 5.48E-05 | 0.128901 | 2.46 |
| FA | 4 | 17.24217 | 4.310543 | 10131.27 | 2.46 |
| FB | 5 | 101.2756 | 20.25512 | 47606.55 | 2.3 |
| Comb(A*B) | 29 | 1.190335 | 0.041046 | 96.4724 | 1.63 |
| Error/Res | 107 | 0.045525 | 0.000425 | | |
| LSD | 0.02583 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.25:** Effect of refrigerated storage (0°C) on fat content of emulsion sausages incorporated with different levels of whey protein concentrate

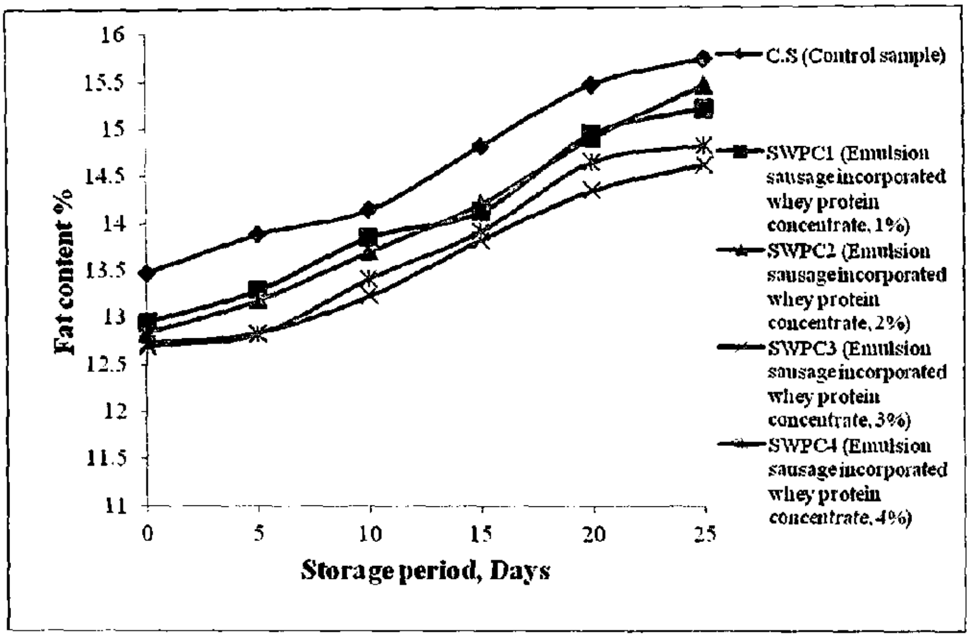
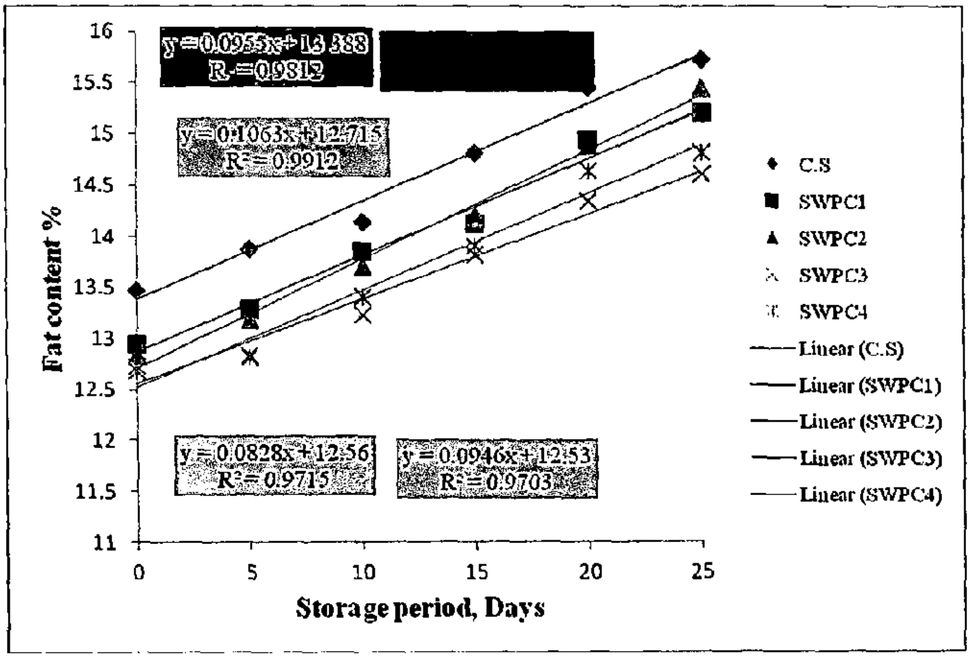



**Fig 4.26:** Regression analysis of fat content of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)



### :.2.6 Thiobarbituric acid number

Thiobarbituric acid (TBA) number is important relevant characteristics of :at products that indicate the oxidation state and later stage, the rancidity of the ›ducts. The emulsion sausages after preparation were packed in LDPE film under ɔospheric packaging system. The samples contained sufficient fat and therefore nples might be oxidized by atmospheric oxygen and may lead to develop warm :r flavour (WOF). TBA measurements have been frequently found to give useful relation with sensory scores, in looking at the development of WOF in cooked ats (Poste *et al.*, 1986). TBA number was determined as mg of malonaldehyde/kg.

ilonaldehyde produced as a result of fat oxidation of fat and its reaction with TBA gent to produce coloured complex with absorption max/min 530-532 nm. The red ;ment produced is the reaction product obtained from condensation of two moles of A reagent with one mole of malonaldehyde (Sinnhuber *et al.*, 1958).

**Emulsion sausage incorporated with protein concentrate**

Tables 4.44 and Figure 4.31 presents the TBA number (mg/kg of .lonaldehyde) of emulsion sausages samples during refrigerated storage. The values TBA number were found to increase consistently during refrigerated storage. The JOVA results (Table 4.45) indicated that treatment of whey protein concentrate at levels of (1, 2, 3 and 4%) significantly ($p<0.05$) affected TBA number of emulsion ısages. The linear regression graphs of these plots/figures have been shown in ;ure 4.32. The regression lines of samples incorporated at the levels of 1, 2, 3 and › of whey protein concentrate have been shown by different colour in Figure 4.32. e equations of regression lines and correlation coefficient have been depicted on regression graph. The positive sign in the equation shows that there was ıtinuous increase of TBA number during storage. The values of correlation ɜfficient ($R^2$) were respectively 0.9377, 0.9559, 0.9721 and 0.9896. The values of were very near to 1. Thus the graph may be approximated to a straight line and ear relation well fits between the storage period and TBA number. There is :relation between sensory attribute (flavour) and TBA number of mat and meat ›ducts. Greene and Cumuze (1982) investigated the relation between TBA numbers 1 assessed oxidized flavour in cooked beef. On the basis of panellists evaluation :y reported that the range of TBA number for which these panellist as a group first detected a difference in intensity of oxidized flavour was 0.6-2.0. Studies of Tarladgis *et al.*, (1960) also showed that the intensity of rancid odour detected in meat sample in close range 0.5-1.0. It can be concluded that TBA test can be used to follow oxidation in muscles foods, although the test should be accompanied frequently by corresponding evaluation with trained sensory panel in studies of WOF in meat (Igene *et al.*, 1979). Sato and Hagarty (1971) reported the effect of poly phosphates in preventing or delaying rancidity in cooked meat.

**b) Emulsion sausage incorporated with protein isolate**

In fresh condition the samples of emulsion sausage, control and treated with whey protein isolate at the levels of 1, 2, 3 and 4% respectively had TBA number in the range of 0.206 and 0.230 mg/kg of malonaldehyde (Table 4.46 and Figure 4.33). The ANOVA (Table 4.47) results indicated there was a significant ($p<0.05$) increase in TBA number during refrigerated storage. After 25 days of storage, samples TBA values of all samples were in the range 0.590-0.639. Ahmad *et al.*, (2005) and Coskuner *et al.*, (2010) claimed that TBA number of semi dry fermented sausages increased during refrigerated storage. Ali (2011) asserted that TBA values of ground beef increased gradually and significantly ($p<0.05$) during storage period. Similar results found by Ahmad *et al.*, (2012) for buffalo meat fermented sausage.

The equation of regression lines and correlation coefficient of all samples with four levels of whey protein isolate have been shown on the regression graph (Fig 4.34). The positive sign in the coefficients of x explains that there was constant increase of TBA values during storage. The values of $R^2$ for all samples at four levels of whey protein isolate were between 0.918-0.996. The values of $R^2$ were very near to 1, so the profile of TBA number during refrigerated storage graph may be represented by a straight line and linear relation well fits between storage period and TBA number.

**c) Emulsion sausage incorporated with whey protein powder**

Table 4.48 and Figure 4.35 represents the results of TBA number analysis of emulsion sausages incorporated with different levels (1, 2, 3 and 4 %) of whey protein powder during refrigerated storage (0°C). TBA number values were between o.195-0.208 mg/kg of malonaldehyde of all sausages. Refrigerated storage significantly ($p<0.05$) increased the TBA number of all samples. On 25$^{th}$ day of storage,sausage samples (both controlled and treated ) had TBA number values in the range of 0.545 – 0.605 mg/kg of malonaldehyde. Bhaskar *et al.*, (2009) explained that mean values of TBA number of the pork sausage incorporated with low, medium and high calcium milk was found to be significantly ($P<0.01$) increase as the storage period increased up to 15 days. This increase in TBA values might be due to a concomitant increase of oxidation of fatty acids and lipid peroxidation during storage. These results match with those observed by Singh and Verma (2000) in chicken meat patties, and Bhattacharyya *et al.*, (2013) in duck sausages. The ANOVA results showed that these results statistically significant ($p<0.05$) for TBA number of emulsion sausages incorporated with whey protein products (Table 4.49).

Figure 4.3 shows the regression analysis of TBA number of emulsion sausages during refrigerated storage at (0°C) incorporated with different levels (1, 2, 3 and 4%) of whey protein powder. The equations of regression and correlation coefficient ($R^2$) have been shown on the regression graph. The positive sign in the coefficients of x explains that there was constant increased of TBA number during refrigerated storage. Values of $R^2$ were very close to 1 and the graph may be approximated to a straight line and linear relation well fits between storage period and TBA number values.

**Table 4.44:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample code | TBA Number mg of malonaldehyde/kg | | | | | |
|---|---|---|---|---|---|---|
| | Storage period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 0.179± 0.004ac | 0.205± 0.005 | 0.265± 0.007 | 0.325± 0.004 | 0.417± 0.004 | 0.589± 0.009d |
| **$Swpc_1$** | 0.191± 0.003b | 0.212± 0.003 | 0.287± 0.009 | 0.365± 0.007 | 0.427± 0.006 | 0.598± 0.005d |
| **$Swpc_2$** | 0.175± 0.005ac | 0.207± 0.003 | 0.286± 0.010 | 0.308± 0.003 | 0.410± 0.004 | 0.516± 0.002e |
| **$Swpc_3$** | 0.174± 0.004ac | 0.223± 0.005 | 0.305± 0.005 | 0.342± 0.004 | 0.428± 0.003 | 0.549± 0.005e |
| **$Swpc_4$** | 0.180± 0.006c | 0.240± 0.005 | 0.317± 0.005 | 0.401± 0.002 | 0.515± 0.005 | 0.610± 0.002f |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$); Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.45:** ANOVA of TBA number of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 2.759368 | 0.018519 | | |
| Replicate | 4 | 0.000172 | 4.3E-05 | 1.222202 | 2.46 |
| FA | 4 | 0.062003 | 0.015501 | 440.9241 | 2.46 |
| FB | 5 | 2.651128 | 0.530226 | 15082.45 | 2.3 |
| Comb(A*B) | 29 | 0.042475 | 0.001465 | 41.66215 | 1.63 |
| Error/Res | 107 | 0.003762 | 3.52E-05 | | |
| LSD | 0.007425 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.31:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein concentrate

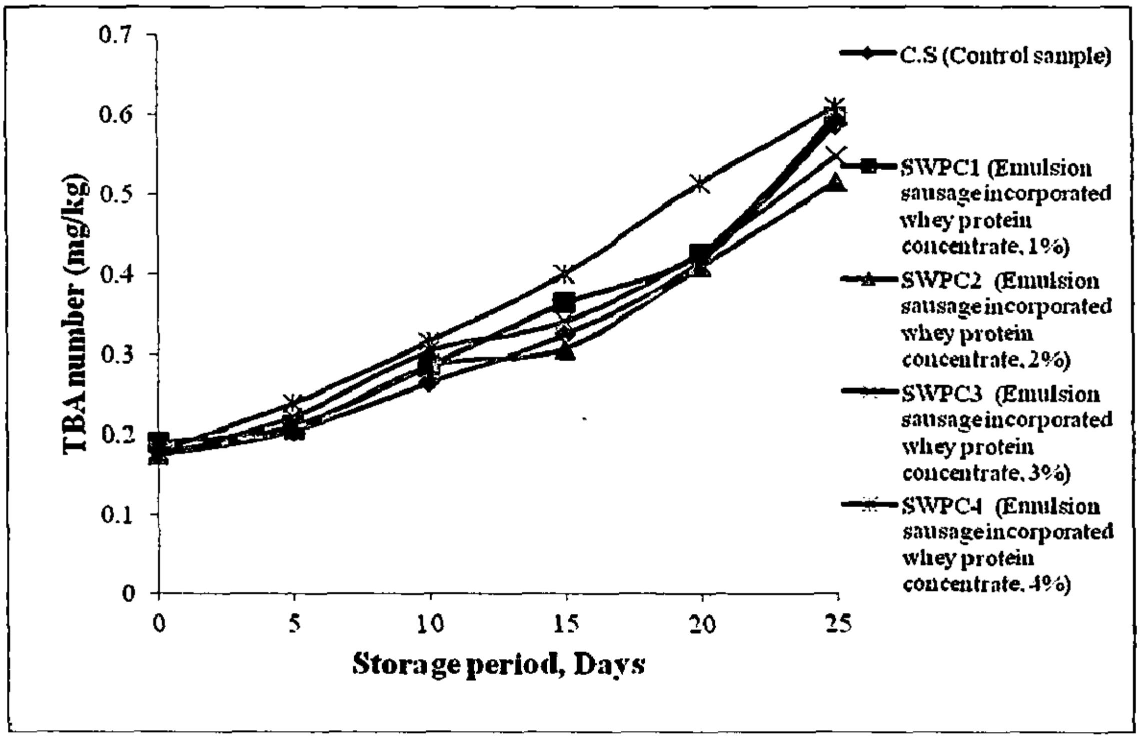


**Fig 4.32:** Regression analysis of TBA value of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage at 0°C

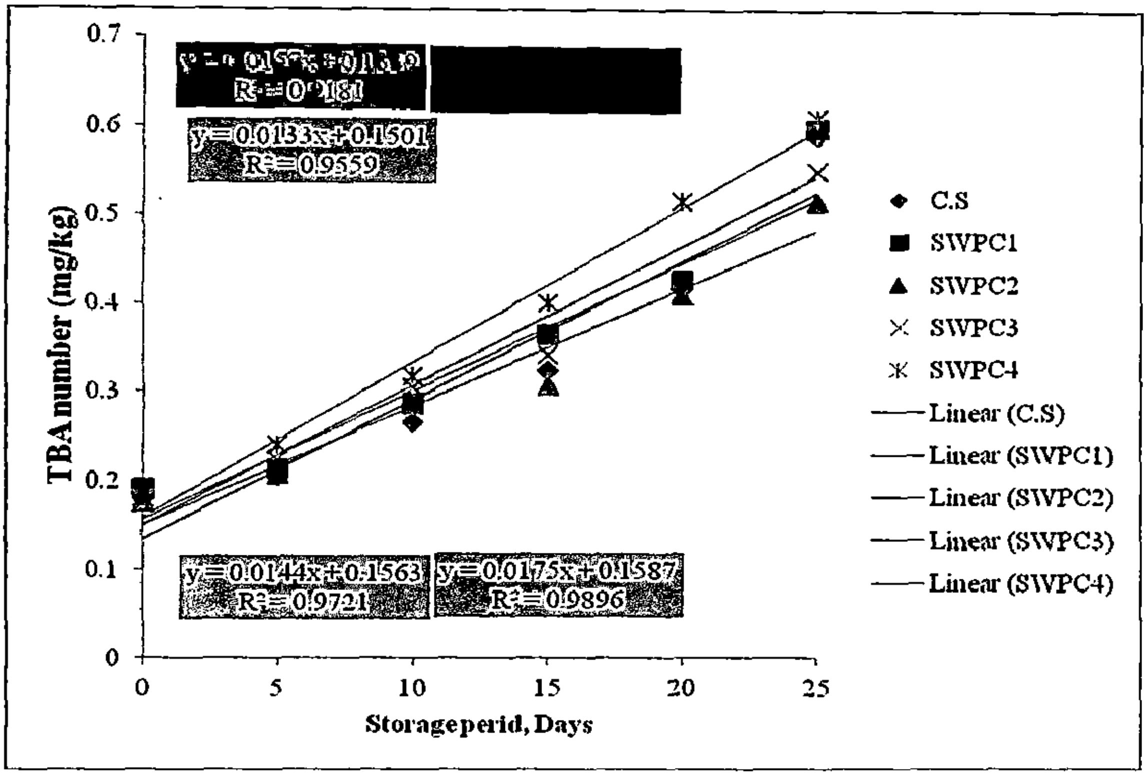



**Table 4.46:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | TBA Number mg of malonaldehyde/kg | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 0.179± 0.004a | 0.205± 0.005 | 0.265± 0.007 | 0.325± 0.004 | 0.417± 0.004 | 0.589± 0.009c |
| Swpi$_1$ | 0.206± 0.007b | 0.294± 0.003 | 0.363± 0.008 | 0.419± 0.006 | 0.506± 0.005 | 0.590± 0.006c |
| Swpi$_2$ | 0.221± 0.002ab | 0.316± 0.004 | 0.384± 0.011 | 0.436± 0.003 | 0.529± 0.004 | 0.601± 0.007e |
| Swpi$_3$ | 0.229± 0.006ab | 0.334± 0.007 | 0.393± 0.004 | 0.484± 0.008 | 0.543± 0.008 | 0.617± 0.002e |
| Swpi$_4$ | 0.230± 0.005ab | 0.345± 0.001 | 0.404± 0.003 | 0.496± 0.004 | 0.574± 0.005 | 0.639± 0.005e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpi$_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.47:** ANOVA of TBA number of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 2.881388 | 0.019338 | | |
| **Replicate** | 4 | 0.00021 | 5.25E-05 | 1.270421 | 2.46 |
| **FA** | 4 | 0.259181 | 0.064795 | 1568.146 | 2.46 |
| **FB** | 5 | 2.570403 | 0.514081 | 12441.56 | 2.3 |
| **Comb(A*B)** | 29 | 0.047383 | 0.001634 | 39.54261 | 1.63 |
| **Error/Res** | 107 | 0.004421 | 4.13E-05 | | |
| **LSD** | 0.00805 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.33:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein isolate

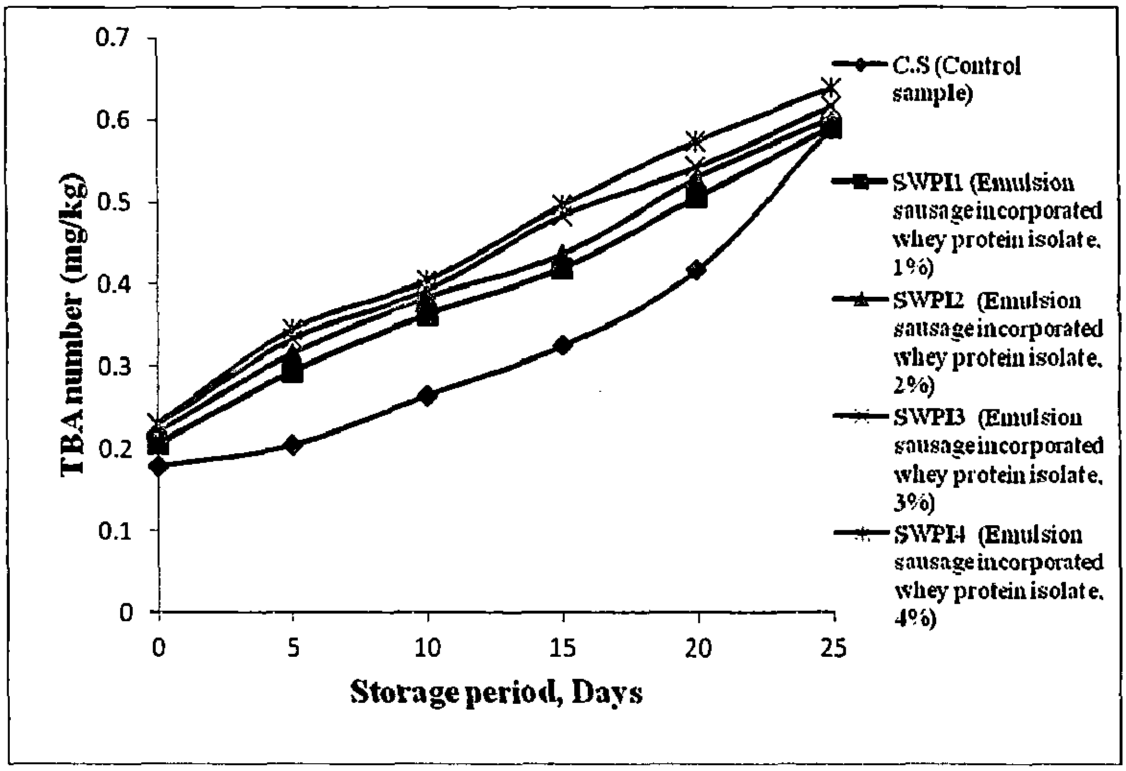


**Fig 4.34:** Regression analysis of TBA value of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

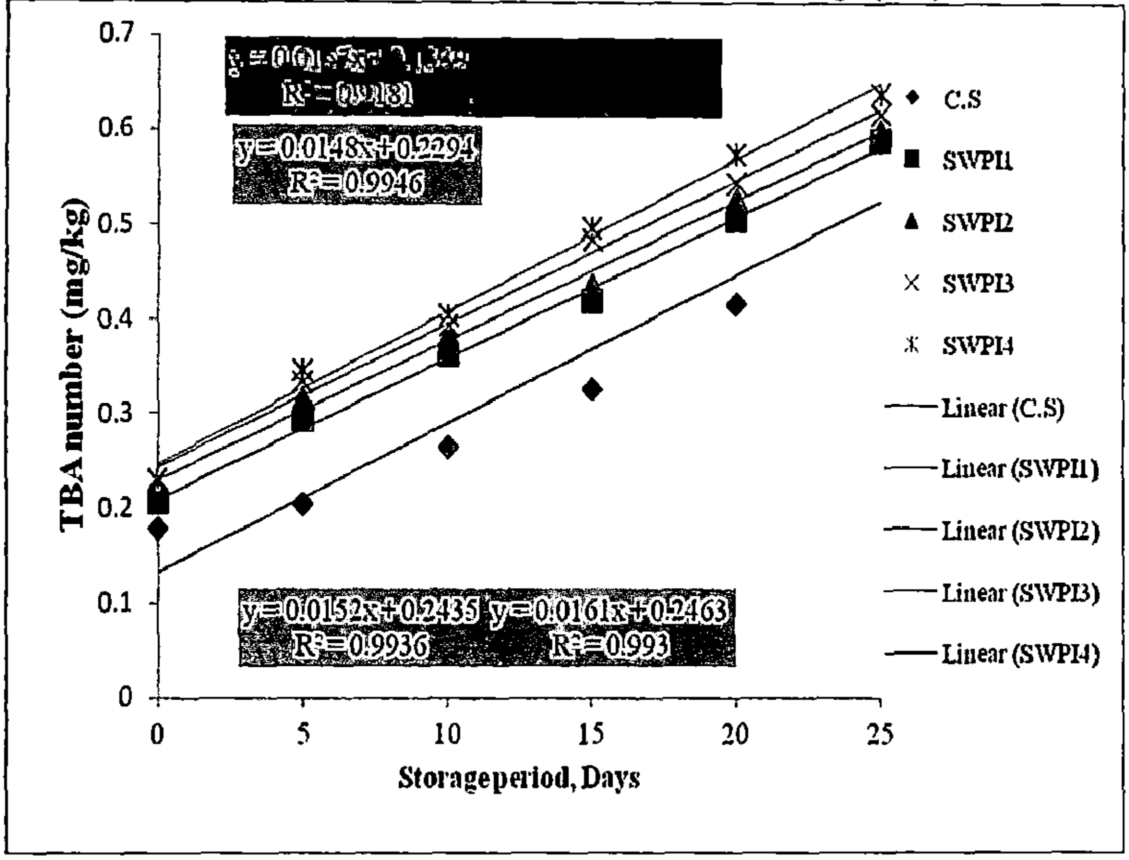



**Table 4.48:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | TBA Number mg of malonaldehyde/kg | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 0.179± 0.004a | 0.205± 0.005 | 0.265± 0.007 | 0.325± 0.004 | 0.417± 0.004 | 0.589± 0.009d |
| $Swpp_1$ | 0.195± 0.004b | 0.251± 0.003 | 0.329± 0.004 | 0.393± 0.001 | 0.474± 0.004 | 0.591± 0.004d |
| $Swpp_2$ | 0.208± 0.006c | 0.290± 0.006 | 0.369± 0.003 | 0.410± 0.002 | 0.492± 0.007 | 0.602± 0.001e |
| $Swpp_3$ | 0.202± 0.002c | 0.286± 0.003 | 0.345± 0.003 | 0.323± 0.002 | 0.421± 0.003 | 0.545± 0.001f |
| $Swpp_4$ | 0.196± 0.008b | 0.249± 0.003 | 0.316± 0.003 | 0.392± 0.002 | 0.471± 0.005 | 0.605± 0.002e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.49:** ANOVA of TBA number of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 2.58372 | 0.01734 | | |
| **Replicate** | 4 | 0.000185 | 4.63E-05 | 1.759131 | 2.46 |
| **FA** | 4 | 0.073189 | 0.018297 | 695.5397 | 2.46 |
| **FB** | 5 | 2.456877 | 0.491375 | 18678.83 | 2.3 |
| **Comb(A*B)** | 29 | 0.05084 | 0.001753 | 66.64133 | 1.63 |
| **Error/Res** | 107 | 0.002815 | 2.63E-05 | | |
| **LSD** | 0.006423 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.35:** Effect of refrigerated storage (0°C) on TBA number of emulsion sausages incorporated with different levels of whey protein powder

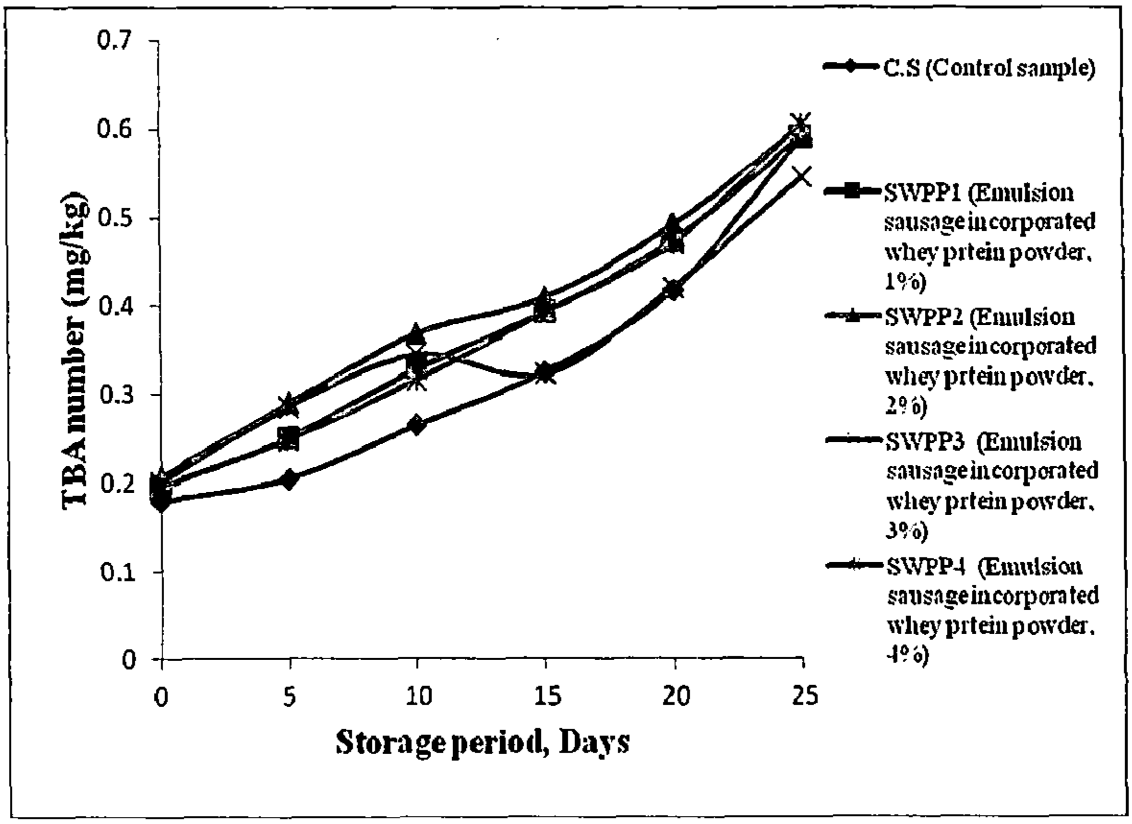
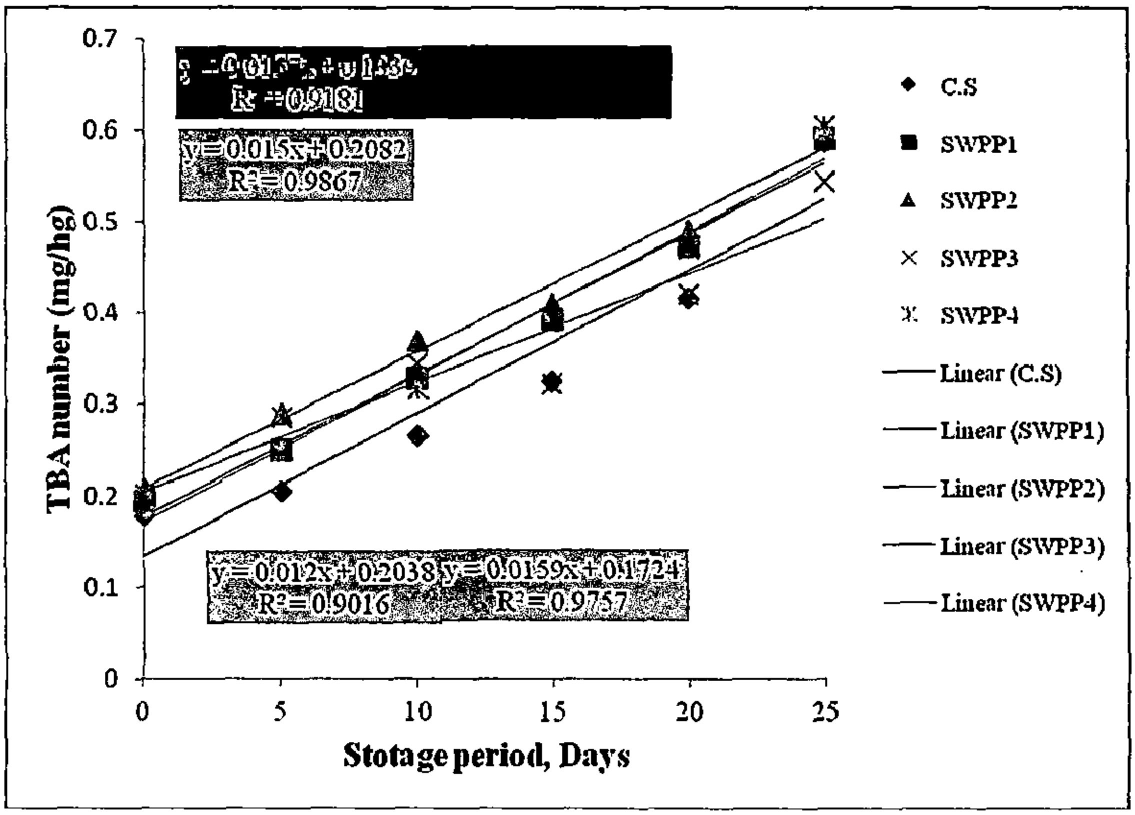


**Fig 4.36:** Regression analysis of TBA value of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)




### 4.2.2.7 Extract release volume

Extract release volume (ERV) appears to have considerable possibilities for assessing the spoilage of beef (Jay, 1964). It has a highly significant correlation with water holding capacity. The procedure is based on measuring the volume of the aqueous filtrate releases from slurry of meat in fixed time. The extract release volume decreases as spoilage progresses and no filtrate is obtained from putrid meat (Singhal, *et al.*, 1997). ERV is useful profile for routine quality control assessment of meat.

**a) Emulsion sausage incorporated with whey protein concentrate**

The extract release volume (ERV) of emulsion sausages including control and incorporated with different levels of whey protein concentrate, have been presented in Table 4.50. Extract release volume of all samples have found in between 34.2 ml and 36.4 ml in fresh condition. ERV of control sample was found to be a little less (32.2ml) as compared to treated sample. The analysis of variance (ANOVA Table 4.51) shows that the use of different levels of whey protein concentrate significantly ($p<0.05$) increased the extract release volume of sausages sample. During refrigerated storage, extract release volume of these samples significantly ($p<0.05$) decreased. The extract release volumes of samples were found in the range of 25.2–27.3 ml after 25 days of storage (Fig. 4.37).

Fig 4.38 shows the linear regression of extract release volume of emulsion sausage samples (control and treated) with whey protein concentrate during refrigerated storage at 0° C. The negative sign on the coefficients of x explains that there was constant decrease of extract release volume during refrigerated storage. The value of correlation coefficient ($R^2$) of all sample were near to 1 and so almost perfect relation existed between storage period and extract release volume.

**b) Emulsion sausage incorporated with whey protein isolate**

The extract release volume of emulsion sausages samples (control and treated) with whey protein isolate have been presented in Table 4.52. The initial extract release volume (ERV) of emulsion sausage treated with levels 1, 2, 3 and 4% whey protein isolate 37.4, 38.8, 35.6 and 36.8 ml respectively. The extract release volume of control sample was 32.2 ml while extract release volume of samples incorporated whey protein isolate were higher than the control sample. Whey proteins isolate significantly ($p<0.05$) increased extract release volume of emulsion sausage as compared the control sample (Table 4.59). Fig 4.39 shows the extract release volume profile of controlled sample and emulsion sausage incorporated with different levels (1, 2, 3 and 4%) of whey protein isolate. During refrigerated storage, extract release volume of these samples was found to significantly ($p<0.05$) decrease. Therefore the ERV of samples were found 24.4 ml for control sample and 26.4, 27.6, 25.3 and 25.9 ml respectively for treated samples on the 25th day of storage.

Fig 4.40 shows the linear regression analysis of extract release volume of emulsion sausage samples (control and treated with 1, 2, 3 and 4% of WPI) during storage at 0° C. The negative sign in the coefficients of x explains that there was constant decrease of ERV during refrigerated storage. The correlation coefficient values explain the correlation between ERV of samples and storage period (days). The decreasing nature of extract release volume with storage (days) time was linear and it is perfect at $R^2=1$. The values of $R^2$ for all five samples were in between 0.9142-0.9924, which shows that correlations are almost perfect and the graph may be approximated to a straight line.

**c) Emulsion sausage incorporated with whey protein powder**

Table 4.54 presents the results of extract release volume of buffalo meat emulsion sausage samples with different levels of (1, 2, 3 and 4%) of whey protein powder under refrigerated storage at 0° C. The extract release volume of all samples of sausages were found in the range of 33.4 -37.2 ml in fresh condition. During refrigerated storage, ERV of sausage samples were found to decrease significantly ($p<0.05$). On 25$^{th}$ day of storage, the extract release volume of samples were found 24.4 ml for control sample and 25.5, 26.2, 27.3 and 26.1ml respectively for treated emulsion sausage at the level 1, 2, 3 and 4% whey protein powder. It was noticed from the interpretation of extract release volume, that quality of emulsion sausages samples incorporated with whey protein products was found to be better as compared to control. The ANOVA showed that these results statistically were significant ($p<0.05$) for ERV of emulsion sausages incorporated with whey protein powder, but the levels of 2 and 4 % WPP treatment were not significantly ($p<0.05$) affected (Table 4.55). Figure 4.41 shows the extract release volume loss behavior of emulsion sausages samples during refrigerated storage at 0° C.

Fig 4.42 shows the regression analysis of extract release volume of emulsion sausages samples (both control and treated with 1, 2, 3 and 4%, of Whey protein powder) during refrigerated storage at 0° C. The equations of regression and correlation coefficient ($R^2$) have been shown in the regression graph. The values of $R^2$ for all samples were between 0.950-0.995, which shows that relation between ERV and storage period (days) are almost perfect and the graph may be approximated to a straight line. Values of $R^2$ were very close to 1 and hence the perfect linear relation was holding for consistent reduction in extract release volume during refrigerated storage.

**Table 4.50:** Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Extract release volume ml | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 32.2± 0.447a | 31.6± 0.894 | 30.2± 0.570 | 28.5± 0.114 | 26.4± 0.296 | 24.4± 0.250f |
| **$Swpc_1$** | 34.2± 0.836b | 32. 2± 0.836 | 32.0± 0.707 | 29.3± 0.282 | 27.7± 0.559 | 26.5± 0.360g |
| **$Swpc_2$** | 35.2± 0.836c | 33.8± 0.570 | 30.2± 0.836 | 30.0± 0.610 | 27.3± 0.311 | 25.2± 0.230h |
| **$Swpc_3$** | 36.4± 0.894d | 33.7± 0.758 | 30.2± 0.758 | 28.1± 0.502 | 28.3± 0.336 | 26.3± 0.356i |
| **$Swpc_4$** | 36.2± 0.836e | 33.0± 0.790 | 29.8± 0.836 | 27.8± 0.570 | 27.8± 0.739 | 27.3± 0.342j |

*Values are mean of five replicates ±SD; Means with different letters in a column differ significantly* ($p<0.05$), Cs= Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.51:** ANOVA of extract release volume of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 1636.69 | 10.9845 | | |
| **Replicate** | 4 | 6.358933 | 1.589733 | 3.660615 | 2.46 |
| **FA** | 4 | 42.92427 | 10.73107 | 24.71 | 2.46 |
| **FB** | 5 | 1465.828 | 293.1655 | 675.0605 | 2.3 |
| **Comb(A*B)** | 29 | 81.47013 | 2.809315 | 6.468897 | 1.63 |
| **Error/Res** | 107 | 46.468 | 0.43428 | | |
| **LSD** | 0.82524 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

Fig 4.37: Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein concentrate

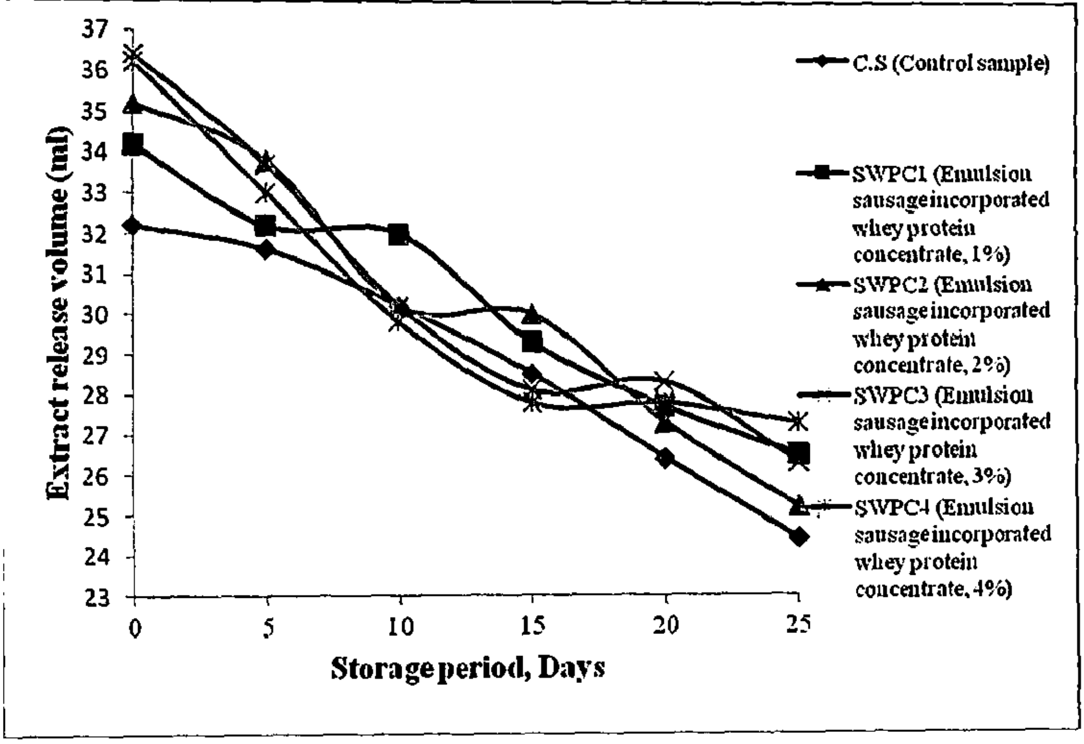



Fig 4.38: Regression analysis of extract release volume of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

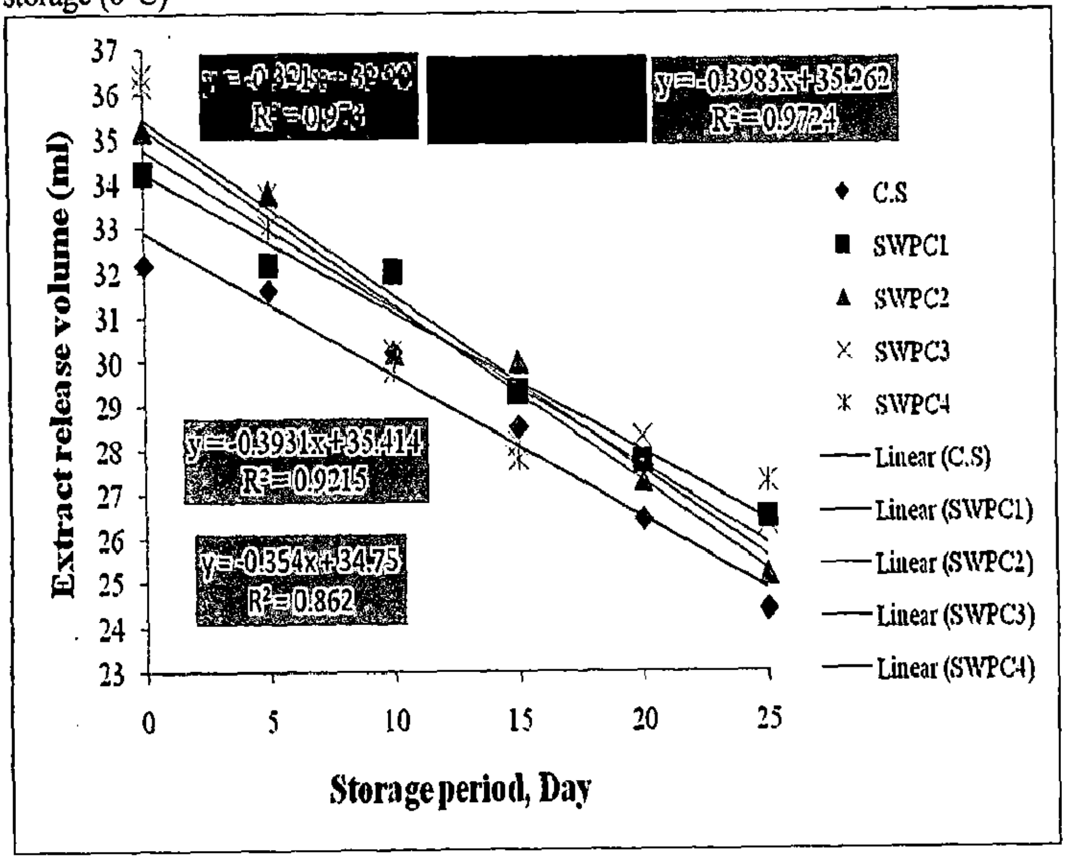


**Table 4.52:** Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Extract release volume ml | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 32.2± 0.447a | 31.6± 0.894 | 30.2± 0.570 | 28.5± 0.114 | 26.4± 0.296 | 24.4± 0.250f |
| **$Swpi_1$** | 37.4± 0.894b | 36.2± 0.277 | 31.6± 0.418 | 29.8± 0.594 | 27.6± 0.421 | 26.4± 0.234g |
| **$Swpi_2$** | 38.8± 0.836c | 34.6± 0.415 | 31.2± 0.273 | 30.4± 0.549 | 29.1± 0.741 | 27.6± 0.415h |
| **$Swpi_3$** | 35.6± 0.894d | 33.5± 0.370 | 31.4± 0.418 | 30.2± 0.758 | 27.8± 0.570 | 25.3± 0.207i |
| **$Swpi_4$** | 36.8± 0.836e | 33.8± 0.622 | 30.7± 0.563 | 30.0± 0.790 | 28.6± 0.418 | 25.9± 0.676j |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs= Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.53:** ANOVA of extract release volume of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 2094.878 | 14.05958 | | |
| **Replicate** | 4 | 3.942267 | 0.985567 | 2.686083 | 2.46 |
| **FA** | 4 | 149.6169 | 37.40423 | 101.9423 | 2.46 |
| **FB** | 5 | 1836.635 | 367.3269 | 1001.12 | 2.3 |
| **Comb(A*B)** | 29 | 69.36627 | 2.39194 | 6.519042 | 1.63 |
| **Error/Res** | 107 | 39.26 | 0.366916 | | |
| **LSD** | 0.75854 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.39:** Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein isolate

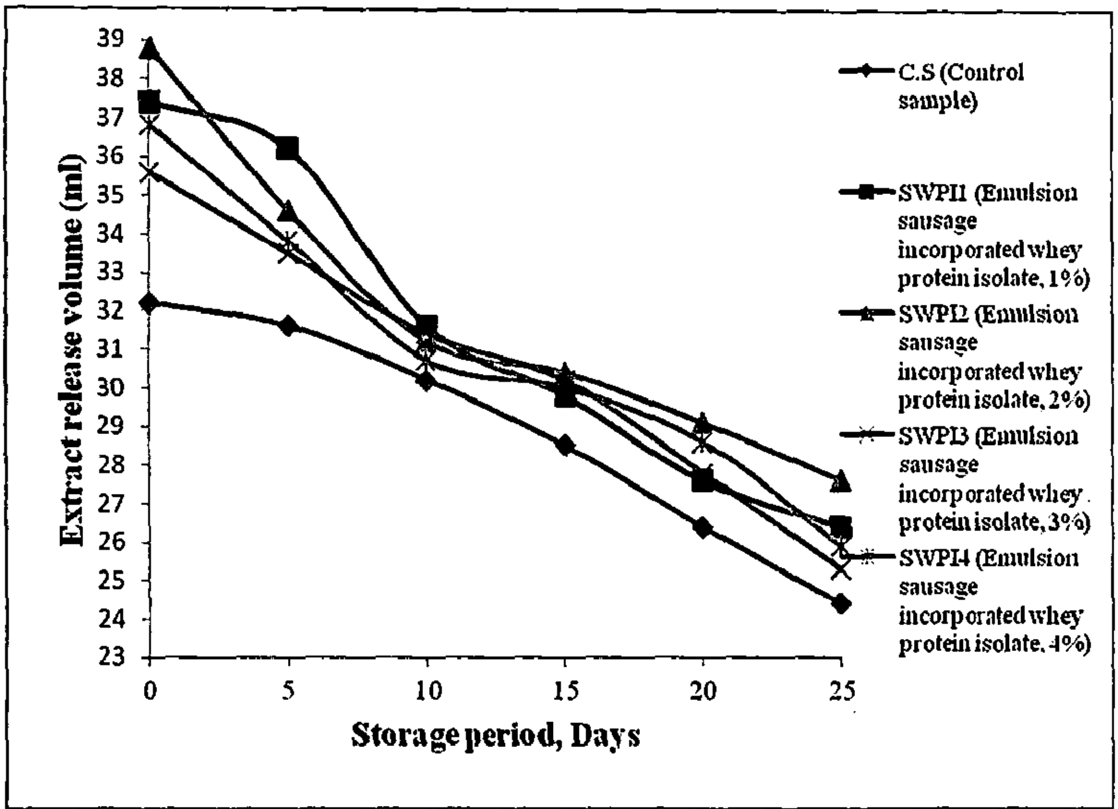



**Fig 4.40:** Regression analysis of extract release volume of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

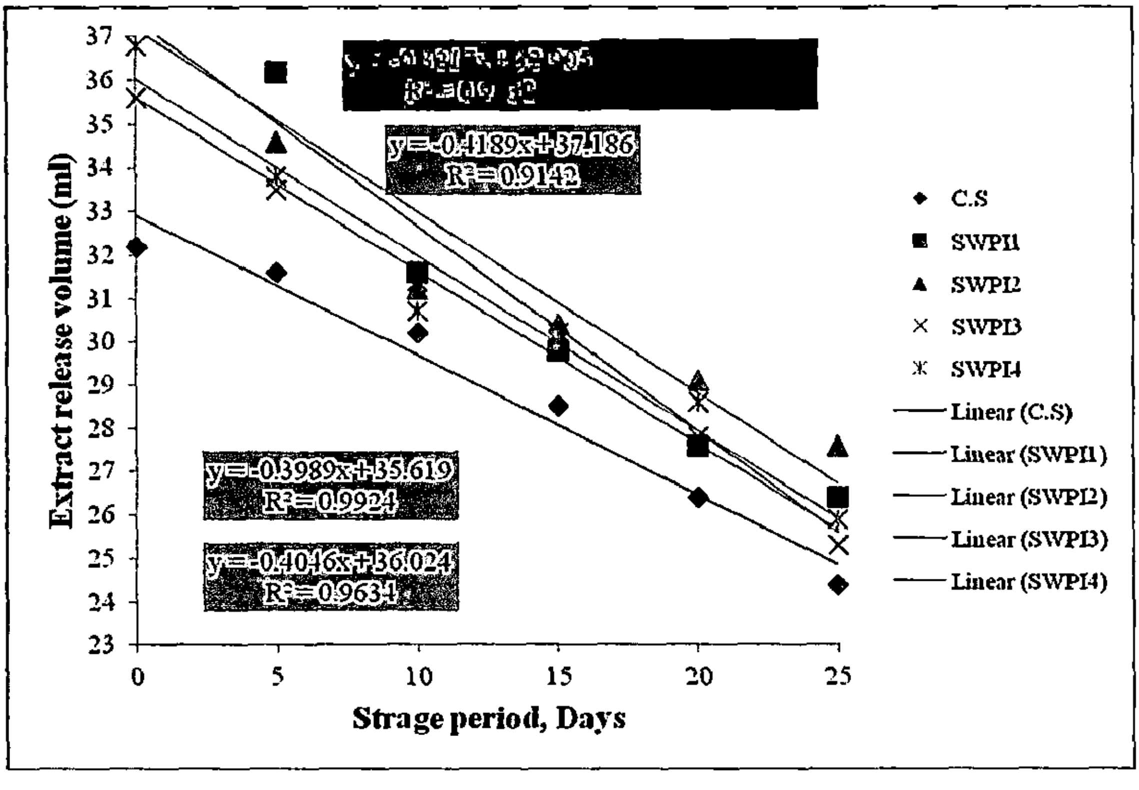


**Table 4.54:** Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Extract release volume ml | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 32.2± 0.447a | 31.6± 0.894 | 30.2± 0.570 | 28.5± 0.114 | 26.4± 0.296 | 24.4± 0.250f |
| **$Swpp_1$** | 33.4± 0.894b | 31.6± 0.651 | 30.2± 0.212 | 29.3± 0.308 | 28.5± 0.396 | 25.2± 0.426g |
| **$Swpp_2$** | 37.2± 0.836c | 35.5± 0.426 | 33.2± 0.260 | 31.3± 0.370 | 28.7± 0.465 | 26.2± 0.234h |
| **$Swpp_3$** | 36.2± 0.836d | 35.1± 0.741 | 32.3± 0.740 | 30.5± 0.350 | 28.2± 0.277 | 27.3± 0.343i |
| **$Swpp_4$** | 36.6± 0.547e | 34.9± 0.593 | 31.6± 0.390 | 29.1± 0.230 | 27.1± 0.216 | 26.1± 0.130h |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.55:** ANOVA of extract release volume of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 1862.888 | 12.50261 | | |
| **Replicate** | 4 | 0.505333 | 0.126333 | 0.444426 | 2.46 |
| **FA** | 4 | 187.834 | 46.9585 | 165.1946 | 2.46 |
| **FB** | 5 | 1578.8 | 315.7599 | 1110.807 | 2.3 |
| **Comb(A*B)** | 29 | 65.8388 | 2.270303 | 7.986667 | 1.63 |
| **Error/Res** | 107 | 30.416 | 0.284262 | | |
| **LSD** | 0.667658 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

Fig 4.41: Effect of refrigerated storage (0°C) on extract release volume of emulsion sausages incorporated with different levels of whey protein powder

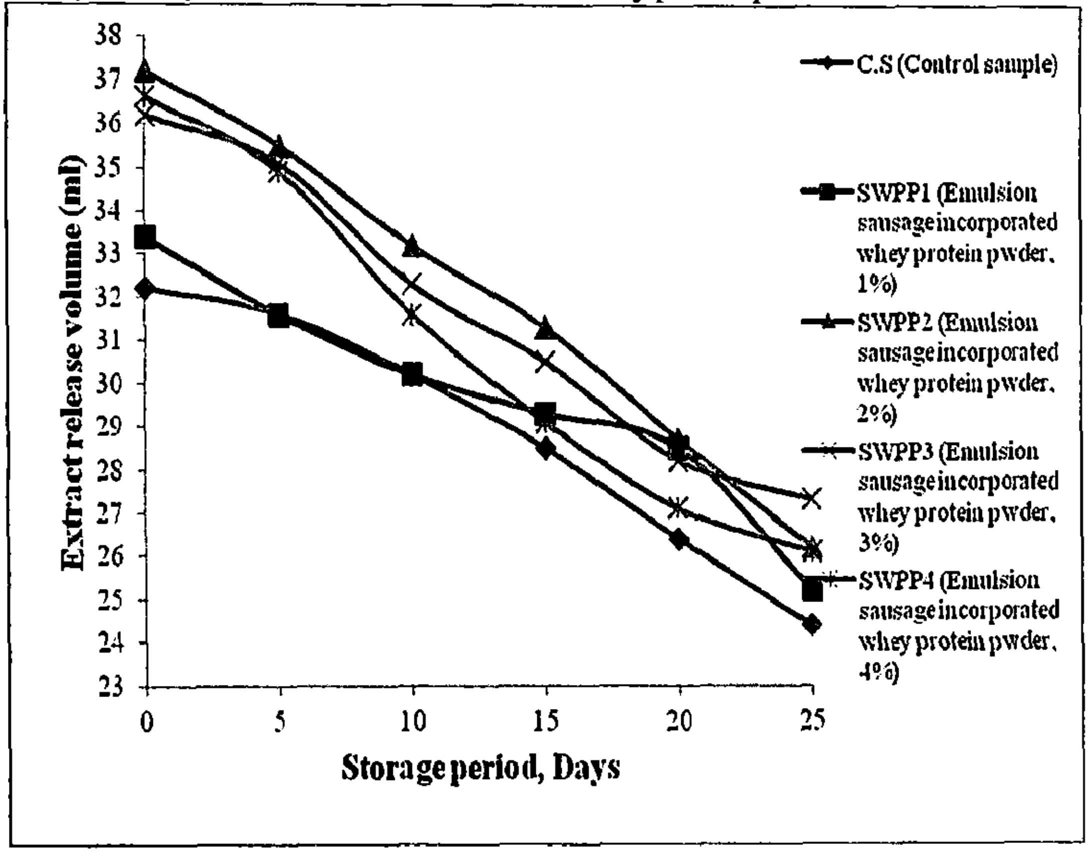
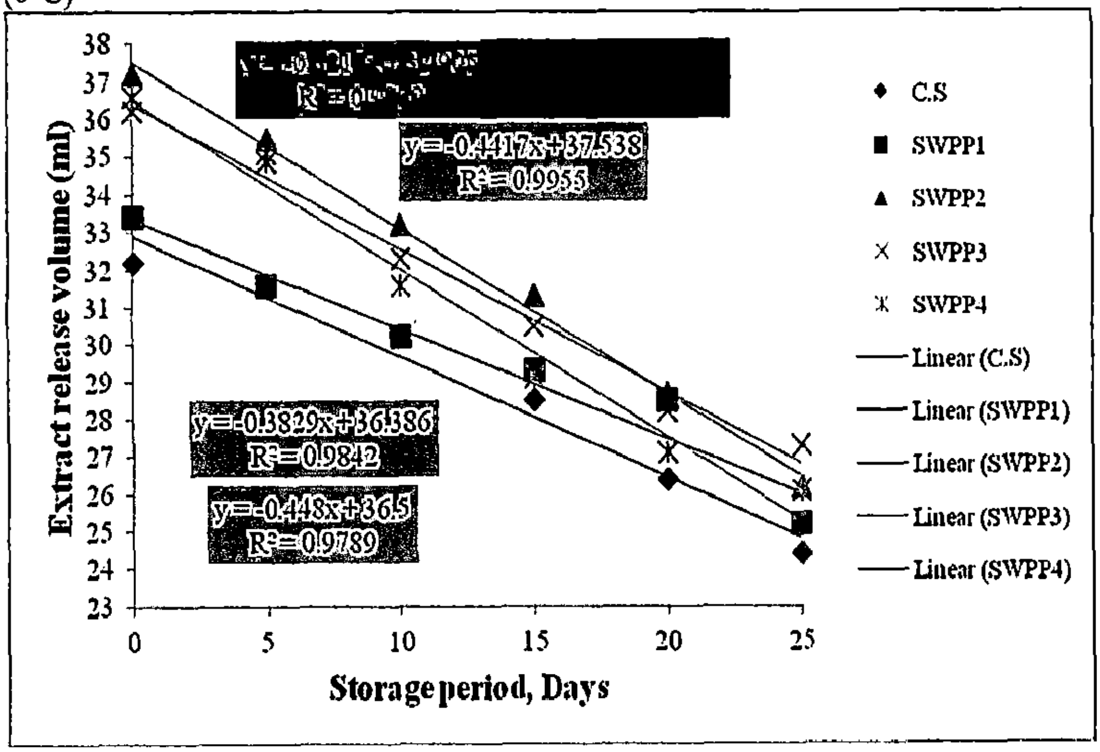



Fig 4.42: Regression analysis of extract release volume of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.2.8 Water holding capacity

Water holding capacity (WHC) has been defined as the ability of meat to retain its own water during application of external forces such as cutting, greanding, processing or heating (forrest *et al.*, 1975). It also can be defined as the ability of the postmortem muscle (meat) to retain water even though external pressures (e.g. gravity, heating) are applied to it. The characteristic of WHC is not trivial. Water-holding capacity of meat can also influence processing characteristics. Meat with low water, WHC often tends to produce inferior processed products (Unknown, 2010).

**a) Emulsion sausage incorporated with whey protein concentrate**

The water holding capacity (WHC) of emulsion sausage samples (control and treated) with different levels (1, 2, 3 and 4%) of whey protein concentrate have been presented in Table 4.56. These samples (control and treated) had WHC in the range 71.18 - 71.50% (on the wet basis) in fresh condition. The control samples had WHC value less than of whey protein treated samples. The analysis of variance (ANOVA Table 4.57) showed that the use of different levels of whey protein concentrate significantly ($p<0.05$) increased the water holding capacity of sausages sample. Similar results were found by Sammel *et* al., (2007) who advocated that milk proteins enhance physical properties including gelation, fat emulsification, and water holding capacity. In another study Sammel and Clus (2003) reported whey proteins contain many hydrophilic groups that are exposed upon heating and react with water, thus increasing the meats water holding capacity. Similar results were reported by (Corrales *et al.,* 2004; Hayes *et al.,* 2006; Hughes *et al.,* 1998) who reported that whey protein concentrate (WPC) was employed to increase water holding capacity. During refrigerated storage, water holding capacity of these samples was found significantly ($p<0.05$) decrease (Fig. 4.43). The moisture contents of treated samples were found in the range of 66.20% - 66.72% on the $25^{th}$ days of storage.

Fig 4.44 shows the linear regression of water holding capacity of emulsion sausage incorporated at the levels 1, 2, 3 and 4% of whey proteins concentrate during refrigerated storage at 0° C. The negative sign in the coefficients of x explains that that there was constant decrease of water holding capacity during refrigerated storage. The value of correlation coefficient ($R^2$) of all sample were near to 1 and so almost perfect relation existed between storage period and moisture content.

**)) Emulsion sausage incorporated with whey protein isolate**

Results of water holding capacity of emulsion sausages incorporated with ifferent levels of whey protein isolate (1, 2, 3 and 4%) have been presented in Table .58. The initial WHC of emulsion sausage treated with 1, 2, 3 and 4% of whey rotein isolate was found to be 72.89, 72.14, 72.08 and 72.15% respectively. The VHC of control sample with was 69.69%. It showed that WHC of treated samples vere higher than the control sample. Whey proteins isolate significantly ($p<0.05$) ncreased water holding capacity of emulsion sausage (Table 59). Fig 4.45 exhibits he WHC profile of (controlled and treated) sausage samples during refrigerated torage. During refrigerated storage, water holding capacity of ES significantly p<0.05) decreased. The water holding capacity of control sample was found to be 4.20% while for treated samples it was found to be 67.57, 68.27, 67.29 and 67.55% espectively with different levels 1, 2, 3 and 4 % of whey protein isolate on the 25$^{th}$ lay of storage.

Fig 4.46 shows the linear regression of water holding capacity of treated mulsion sausage samples during refrigerated storage at 0° C. The negative sign on he coefficients of x explains that there was constant decrease of water holding apacity during refrigerated storage. The correlation coefficient values explain the elation between water holding capacity and storage period (days). The decreasing ature of water holding capacity storage time (days) was in fact perfect at $R^2=1$. The alues of $R^2$ for all five samples were in between 0.9565 to 0.9933, which shows lmost as perfect linear relationship graph may be approximated to a straight line. A lecrease in moisture content during storage was the reason for loss in WHC of ausage samples.

**:) Emulsion sausage incorporated with whey protein powder**

Table 4.60 present the results of water holding capacity of buffalo meat mulsion sausages treated with different levels (1, 2, 3 and 4%) of whey protein owder during refrigerated storage at 0° C. The water holding capacities of all amples of emulsion sausages were found in the range of 74.60-75.37% in fresh ondition. Different levels of whey protein powder (1, 2, 3 and 4%) significantly p<0.05) increased the water holding capacity of sausage samples. Many researchers Pietrasik and Duda, 2000; Dexter, *et al.*, 1993; Hung and Zayas, 1992; Lecomte *et ıl*,. 1992) found that adding whey proteins to frankfurter type's meat products ncreased the water holding capacity and resulted high WHC. Similar results were recorded by Serdaroğlu and Özsümer (2003) this research showed that addition of whey powder caused elevation of WHC in range of 73.0% in cooked beef sausages with 20% fat. Result of ANOVA for WHC showed that different levels of whey protein powder significantly ($p<0.05$) increase the WHC of emulsion sausage samples (Table 4.49). During refrigerated storage it was found that water holding capacity of emulsion sausage samples significantly ($p<0.05$) decreased. Control sample had WHC as 64.2% while the WHC of treated sample were respectively 69.47, 68.78, 70.21 and 69.46%. The decrease in water holding capacity was due to evaporation of moisture through the permeable packaging film. Fig 4.47 shows the water holding capacity loss behavior of emulsion sausages samples during refrigerated storage at 0° C.

Fig 4.48 shows the regression analysis of water holding capacity of emulsion sausages samples (control and treated) with whey protein powder at levels 1, 2, 3 and 4% during refrigerated storage at 0°C. The equations of regression and correlation coefficient ($R^2$) have been shown on the regression graph. Values of $R^2$ were very close to 1 and hence the perfect linear relation was holding for consistent reduction in moisture content during refrigerated storage.

**Table 4.56:** Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Water holding capacity % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 69.69± 0.089a | 68.56± 0.044 | 67.72± 0.077 | 66.79± 0.020 | 65.19± 0.044 | 64.20± 0.056f |
| **$Swpc_1$** | 71.22± 0.036b | 70.22± 0.016 | 69.59± 0.042 | 68.91± 0.053 | 67.83± 0.065 | 66.52± 0.058g |
| **$Swpc_2$** | 71.50± 0.246c | 70.24± 0.070 | 69.46± 0.066 | 69.17± 0.043 | 67.76± 0.075 | 66.72± 0.090h |
| **$Swpc_3$** | 71.48± 0.225d | 70.18± 0.038 | 69.77± 0.050 | 68.91± 0.051 | 67.37± 0.094 | 66.27± 0.051i |
| **$Swpc_4$** | 71.18± 0.037e | 70.31± 0.079 | 69.28± 0.043 | 68.70± 0.052 | 67.48± 0.063 | 66.20± 0.063i |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.57:** ANOVA of water holding capacity of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 528.2962 | 3.545612 | | |
| **Replicate** | 4 | 0.056343 | 0.014086 | 1.789984 | 2.46 |
| **FA** | 4 | 103.9854 | 25.99636 | 3303.575 | 2.46 |
| **FB** | 5 | 419.3629 | 83.87258 | 10658.39 | 2.3 |
| **Comb(A*B)** | 29 | 4.105865 | 0.141582 | 17.99196 | 1.63 |
| **Error/Res** | 107 | 0.842 | 0.007869 | | |
| **LSD** | 0.111086 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.43:** Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein concentrate

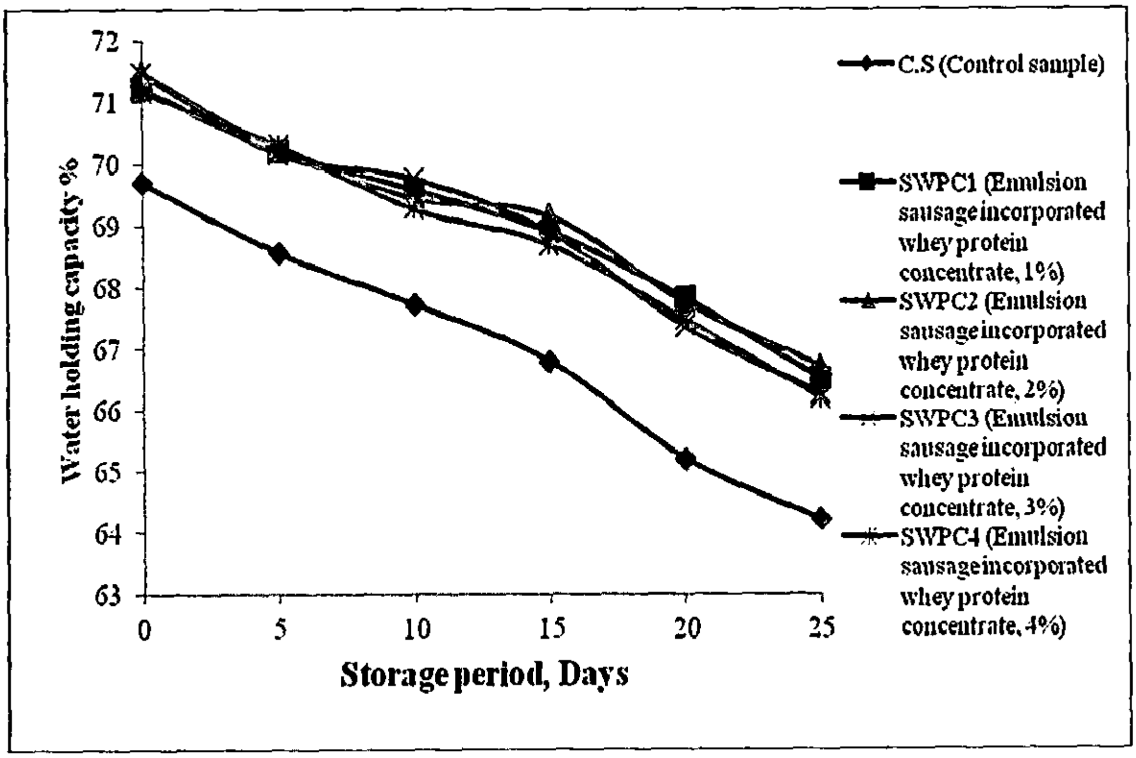


Fig 4.44: Regression analysis of water holding capacity of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

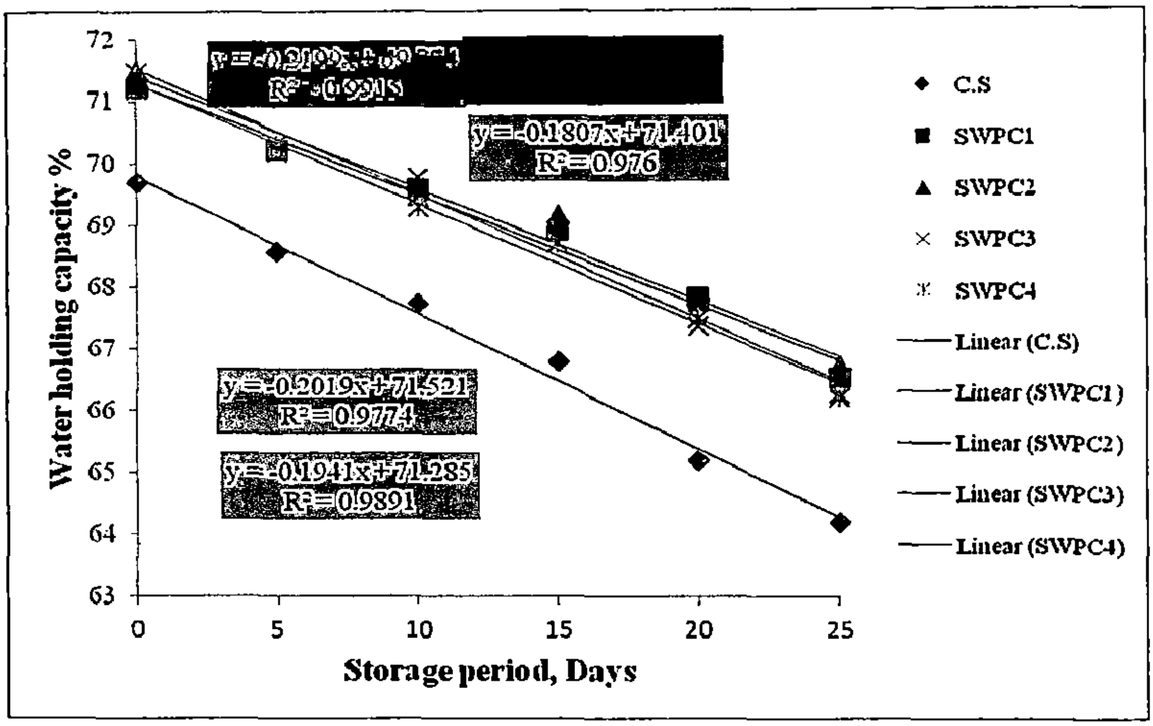



**Table 4.58:** Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Water holding capacity % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 69.69± 0.089a | 68.56± 0.044 | 67.72± 0.077 | 66.79± 0.020 | 65.19± 0.044 | 64.20± 0.056e |
| Swpi1 | 72.89± 0.086b | 72.14± 0.089 | 71.51± 0.095 | 70.36± 0.085 | 68.92± 0.056 | 67.57± 0.088f |
| $Swpi_2$ | 72.14± 0.023c | 71.69± 0.045 | 71.07± 0.039 | 70.62± 0.087 | 69.41± 0.036 | 68.27± 0.095g |
| $Swpi_3$ | 72.08± 0.027d | 71.34± 0.073 | 70.52± 0.048 | 69.43± 0.029 | 68.52± 0.052 | 67.29± 0.093h |
| $Swpi_4$ | 72.15± 0.044c | 71.25± 0.071 | 70.68± 0.049 | 69.22± 0.082 | 68.19± 0.094 | 67.55± 0.074f |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.59:** ANOVA of water holding capacity of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 692.042 | 4.644577 | | |
| **Replicate** | 4 | 0.020767 | 0.005192 | 1.008768 | 2.46 |
| **FA** | 4 | 269.1539 | 67.28848 | 13074.5 | 2.46 |
| **FB** | 5 | 412.2497 | 82.44994 | 16020.45 | 2.3 |
| **Comb(A*B)** | 29 | 10.08767 | 0.347851 | 67.58923 | 1.63 |
| **Error/Res** | 107 | 0.55068 | 0.005147 | | |
| **LSD** | 0.089837 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.45:** Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein concentrate

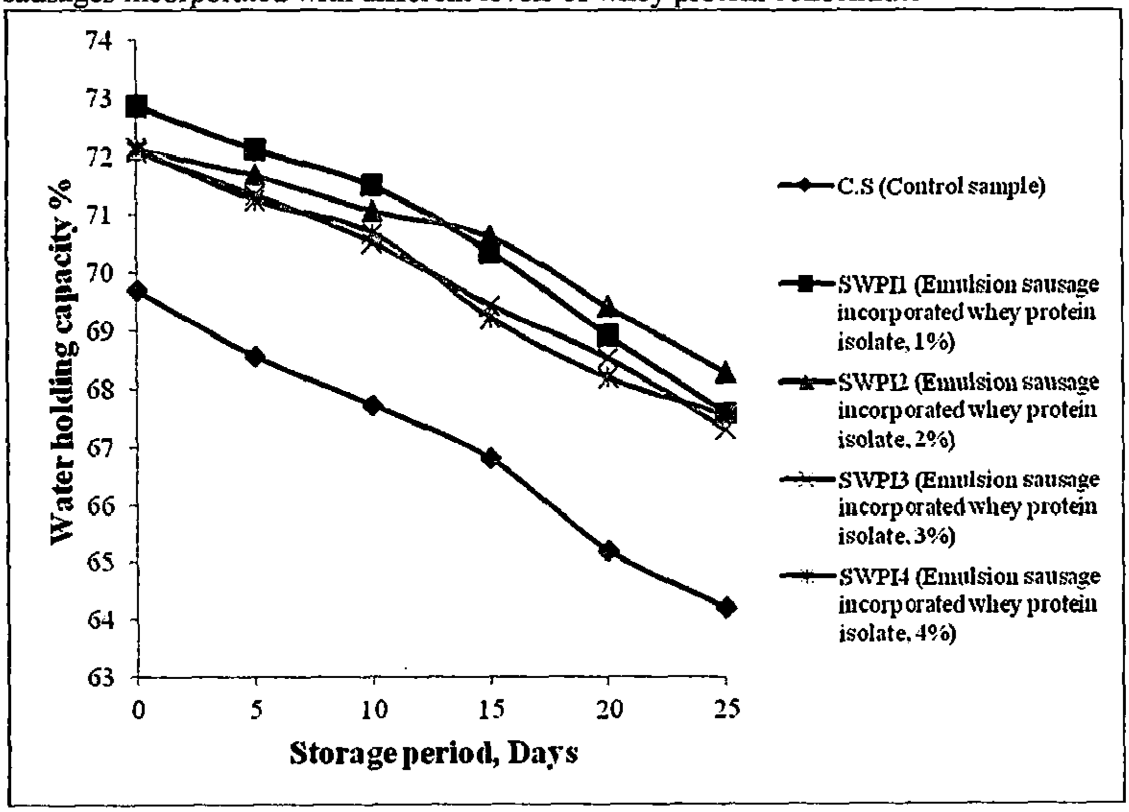


Fig 4.46: Regression analysis of water holding capacity of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

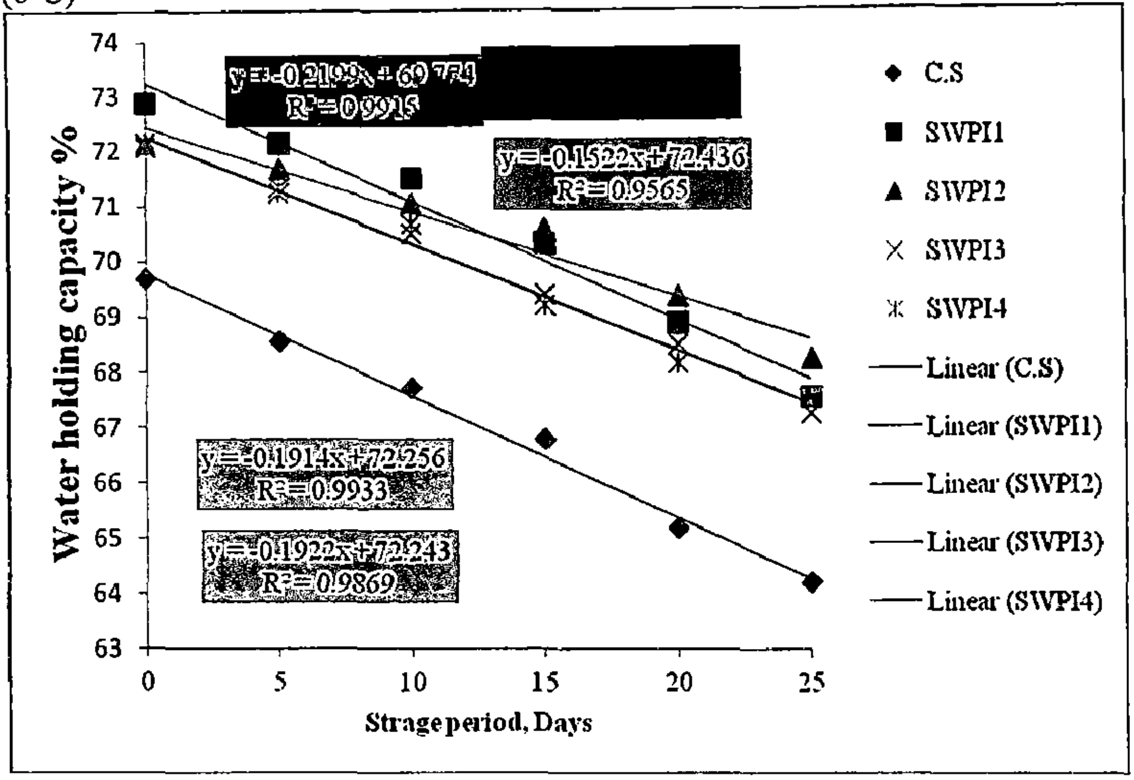



Table 4.60: Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Water holding capacity % | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 69.69± 0.089a | 68.56± 0.044 | 67.72± 0.077 | 66.79± 0.020 | 65.19± 0.044 | 64.20± 0.056f |
| Swpp1 | 75.37± 0.098b | 74.25± 0.054 | 73.18± 0.081 | 72.41± 0.033 | 70.91± 0.041 | 69.47± 0.090g |
| Swpp2 | 74.90± 0.063c | 73.86± 0.040 | 72.24± 0.098 | 71.85± 0.055 | 70.23± 0.082 | 68.78± 0.021h |
| Swpp3 | 75.22± 0.054d | 74.67± 0.080 | 73.44± 0.097 | 72.19± 0.064 | 71.29± 0.064 | 70.21± 0.085i |
| Swpp4 | 74.60± 0.054e | 73.88± 0.064 | 72.82± 0.048 | 71.72± 0.045 | 70.29± 0.050 | 69.46± 0.056g |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.61:** ANOVA of water holding capacity of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 1272.065 | 8.537352 | | |
| **Replicate** | 4 | 0.004709 | 0.001177 | 0.237778 | 2.46 |
| **FA** | 4 | 725.5424 | 181.3856 | 36633.18 | 2.46 |
| **B** | 5 | 541.275 | 108.255 | 21863.51 | 2.3 |
| **Comb(A*B)** | 29 | 4.718209 | 0.162697 | 32.85875 | 1.63 |
| **Error/Res** | 107 | 0.5298 | 0.004951 | | |
| **LSD** | 0.088117 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.47:** Effect of refrigerated storage (0°C) on water holding capacity of emulsion sausages incorporated with different levels of whey protein powder

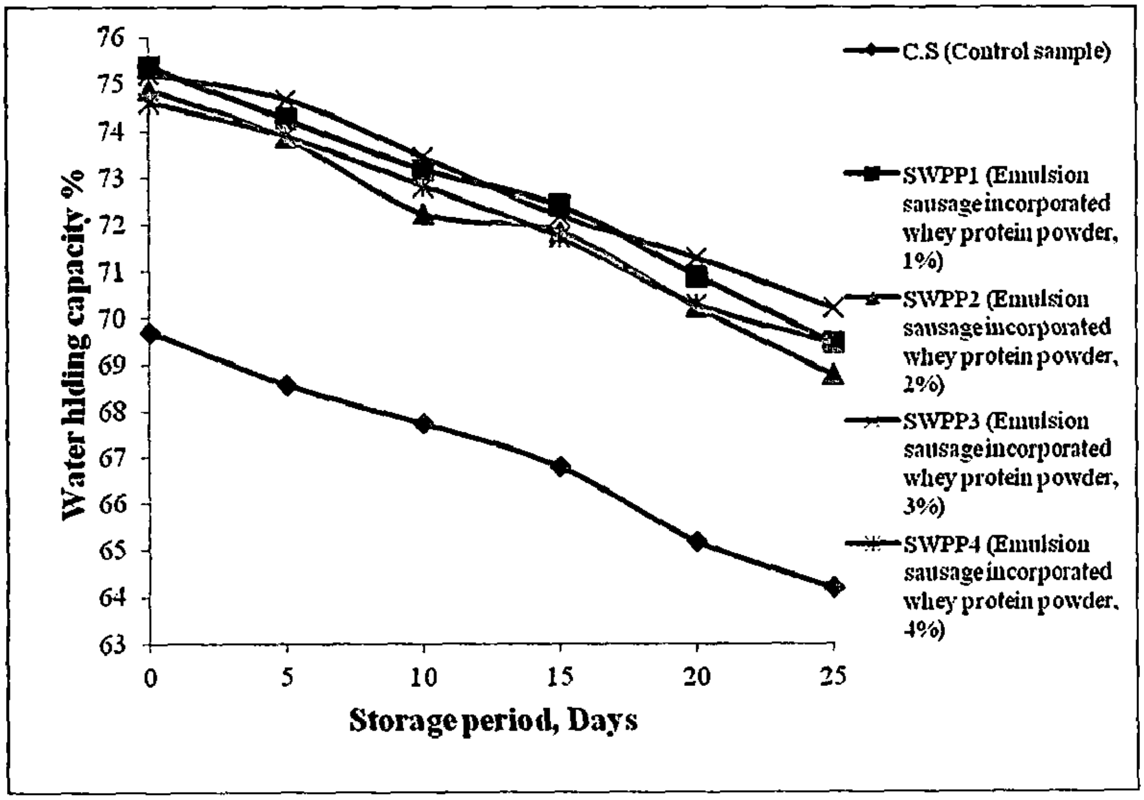
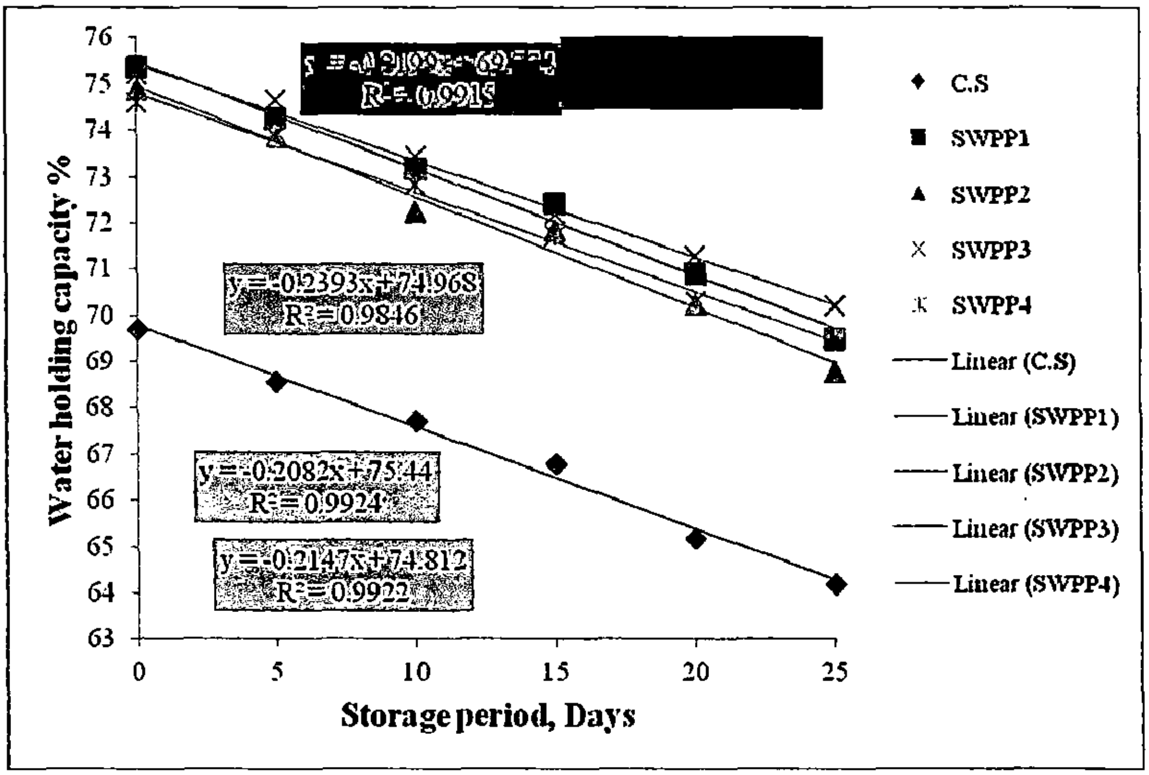


**Fig 4.48:** Regression analysis of water holding capacity of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)




### 4.2.3 Microbiological characteristics of buffalo meat emulsion sausage during refrigerated storage 0°C

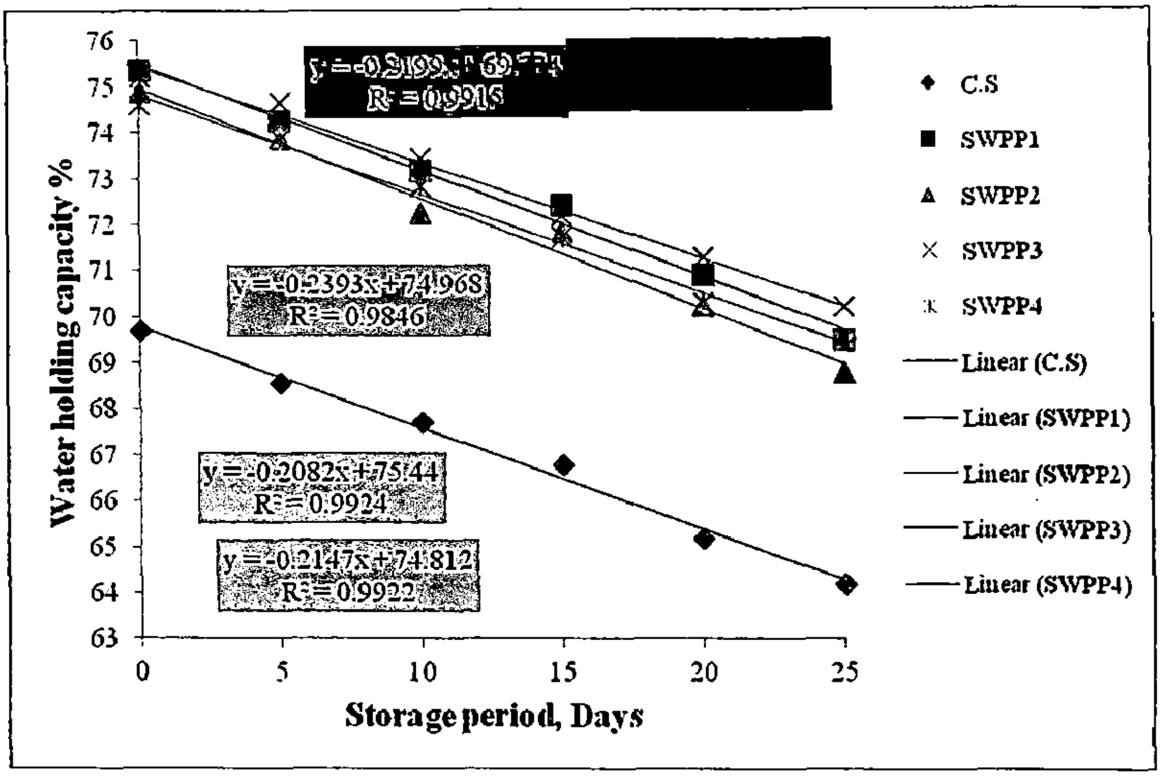
#### 4.2.3.1 Total plate count (TPC)

Buffalo meat emulsion sausages are food with high moisture and good in nutrition and therefore they are center for attraction of microorganism especially bacteria because of favorable pH for their growth. Microbial contamination may be added or reduced at different stages of processing of buffalo sausage. Total plate count of emulsion sausages were evaluated and reported as log (cfu)/g.

Total plate count of samples of emulsion sausages were enumerated in fresh condition and periodically after every 5 days during refrigerated storage at 0°C. Table 4.62, 4.64 and 4.66 presents the microbial profile of emulsion sausages (controlled and treated) during refrigerated storage at 0°C. The ANOVA results (Table 4.63, 4.65 and 4.67) indicated refrigerated storage significantly ($p<0.05$) increased total plate count of emulsion sausages incorporated with whey protein products at 0°C (Figure 4.49, 4.52 and 4.53). The total plate count was found to be between 7.11- 7.39 log cfu/g, 6.74-7.23 log cfu/g and 6.76-7.21 log cfu/g on 25$^{th}$ day of storage for samples prepared with different levels (1, 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder respectively. The end of $25^{th}$ day of refrigerated storage (0°C) total plate count of all sausage samples were found to be in the safe limit (3.32-3.93 log cfu/g). Similar result of TPC were reported by Sachindra *et al.*, (2005), who advocated that total plate counts (log cfu/g) buffalo sausage significantly ($p<0.05$) increased till 35 days of storage with different packaging system (packed under vacuum and nitrogen flush system). But the sausage sample packed under nitrogen atmosphere had more increased value of TPC on compared to vacuum packaging from fourth day onward. Although Bhaskar *et al.*, (2009) also reported total plate count of pork sausages increased significantly ($p<0.01$) and progressively as the storage (7±1°C) period increased. This might be due to the permissive temperature and relative availability of moisture and nutrients for the growth of the aerobes. A similar trend of significant increase in the mean total plate count under refrigerated storage was also observed by Murthy (1986) in pork sausages, Nath *et al.*, (1995) in chicken patties and Rao *et al.*, (1996) in smoked chicken sausages. Ranken and Kill (1993) described that the spoilage condition which are detected when total plate count increase to $10^7$ per g in meat and meat products. The results are also in an agreement with Hytainen *et al.*, (1966) and Essory *et al.*, (1985). Kala *et al.*, (2007) described the total plate count showed an increasing trend for chicken emulsion patties in during the storage. This finding is in agreement with Foster *et al.*, (1977) in ground beef and beef soy patties. Panda (1971) had also reported that incipient of spoilage of meat occurred when aerobic mesophile count of meat samples reduced to log cfu/g.

Meat is rich in nutrition and therefore it is centre of all attraction for micro-organisms especially for bacteria due to their desirable pH for growth. Bacteria even at 0°C are surviving though the rate of growth is slow. Increased microbial population caused the degradation of protein and fat into simpler compounds like fatty acid, amino acid, ammonia, sulphur dioxide and carbon dioxide. Therefore sausage developed off falvour after complete spoilage (Ahmad, 2005). As reported by Brooks *et al.*, (2008) some authors stated that microbial population on raw beef must reach approximately $10^8$ cfu/g to show tackiness when touched, whereas others have claimed that proteolytic changes do not occur until bacterial populations are greater than $3.2\times 10^9$ cfu/cm$^2$ are reached. The ANOVA result indicated that the different levels of whey protein products significantly ($p<0.05$) affected total plate count of emulsion sausages.

**Fig 4.48:** Regression analysis of water holding capacity of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)




### 4.2.3 Microbiological characteristics of buffalo meat emulsion sausage during refrigerated storage 0°C

#### 4.2.3.1 Total plate count (TPC)

Buffalo meat emulsion sausages are food with high moisture and good in nutrition and therefore they are center for attraction of microorganism especially bacteria because of favorable pH for their growth. Microbial contamination may be added or reduced at different stages of processing of buffalo sausage. Total plate count of emulsion sausages were evaluated and reported as log (cfu)/g.

Total plate count of samples of emulsion sausages were enumerated in fresh condition and periodically after every 5 days during refrigerated storage at 0°C. Table 4.62, 4.64 and 4.66 presents the microbial profile of emulsion sausages (controlled and treated) during refrigerated storage at 0°C. The ANOVA results (Table 4.63, 4.65 and 4.67) indicated refrigerated storage significantly ($p<0.05$) increased total plate count of emulsion sausages incorporated with whey protein products at 0°C (Figure 4.49, 4.52 and 4.53). The total plate count was found to be between 7.11- 7.39 log cfu/g, 6.74-7.23 log cfu/g and 6.76-7.21 log cfu/g on 25$^{th}$ day of storage for samples prepared with different levels (1, 2, 3 and 4%) of whey protein concentrate, isolate and whey protein powder respectively. The end of 25th day of refrigerated storage (0°C) total plate count of all sausage samples were found to be in the safe limit (3.32-3.93 log cfu/g). Similar result of TPC were reported by Sachindra *et al.*, (2005), who advocated that total plate counts (log cfu/g) buffalo sausage significantly ($p<0.05$) increased till 35 days of storage with different packaging system (packed under vacuum and nitrogen flush system). But the sausage sample packed under nitrogen atmosphere had more increased value of TPC on compared to vacuum packaging from fourth day onward. Although Bhaskar *et al.*, (2009) also reported total plate count of pork sausages increased significantly ($p<0.01$) and progressively as the storage (7±1°C) period increased. This might be due to the permissive temperature and relative availability of moisture and nutrients for the growth of the aerobes. A similar trend of significant increase in the mean total plate count under refrigerated storage was also observed by Murthy (1986) in pork sausages, Nath *et al.*, (1995) in chicken patties and Rao *et al.*, (1996) in smoked chicken sausages. Ranken and Kill (1993) described that the spoilage condition which are detected when total plate count increase to $10^7$ per g in meat and meat products. The results are also in an agreement with Hytainen *et al.*, (1966) and Essory *et al.*, (1985). Kala *et al.*, (2007) described the total plate count showed an increasing trend for chicken emulsion patties in during the storage. This finding is in agreement with Foster *et al.*, (1977) in ground beef and beef soy patties. Panda (1971) had also reported that incipient of spoilage of meat occurred when aerobic mesophile count of meat samples reduced to log cfu/g.

Meat is rich in nutrition and therefore it is centre of all attraction for micro-organisms especially for bacteria due to their desirable pH for growth. Bacteria even at 0°C are surviving though the rate of growth is slow. Increased microbial population caused the degradation of protein and fat into simpler compounds like fatty acid, amino acid, ammonia, sulphur dioxide and carbon dioxide. Therefore sausage developed off falvour after complete spoilage (Ahmad, 2005). As reported by Brooks *et al.*, (2008) some authors stated that microbial population on raw beef must reach approximately $10^8$ cfu/g to show tackiness when touched, whereas others have claimed that proteolytic changes do not occur until bacterial populations are greater than $3.2\times 10^9$ cfu/cm$^2$ are reached. The ANOVA result indicated that the different levels of whey protein products significantly ($p<0.05$) affected total plate count of emulsion sausages.

Figures 4.50, 4.52 and 4.54 shows the regression analysis of TPC of emulsion sausages during refrigerated storage at 0°C produced by whey protein concentrate, isolate and powder respectively. The equation of regression lines and correlation coefficients were shown on the regression graphs. The positive sign in the coefficients of x explain that there was constant increase in TPC during refrigerated storage. The correlation coefficient values exhibited a linear relation between TPC values and storage days. The increasing nature of TPC with storage time was perfect at $R^2$=1, the values of $R^2$ for all samples were found near 1 which shows that correlation are almost perfect and the graphs may be approximated to a straight line.

**Table 4.62:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | TPC (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 3.32± 0.017a | 3.95± 0.032 | 4.46± 0.013 | 5.12± 0.016 | 5.91± 0.038 | 6.65± 0.027e |
| **$Swpc_1$** | 3.52± 0.016b | 4.65± 0.007 | 5.12± 0.011 | 5.97± 0.016 | 6.54± 0.027 | 7.12± 0.019f |
| **$Swpc_2$** | 3.60± 0.015c | 4.51± 0.018 | 5.22± 0.015 | 6.09± 0.024 | 6.83± 0.027 | 7.33± 0.035g |
| **$Swpc_3$** | 3.64± 0.014c | 4.66± 0.014 | 5.45± 0.013 | 6.33± 0.021 | 6.94± 0.032 | 7.39± 0.020g |
| **$Swpc_4$** | 3.72± 0.015d | 4.50± 0.015 | 5.32± 0.008 | 5.94± 0.026 | 6.64± 0.028 | 7.11± 0.039f |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.63:** ANOVA of total plate count of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 237.4777 | 1.59381 | | |
| **Replicate** | 4 | 0.002309 | 0.000577 | 0.438367 | 2.46 |
| **FA** | 4 | 12.33488 | 3.083719 | 2341.456 | 2.46 |
| **FB** | 5 | 223.1355 | 44.6271 | 33885.18 | 2.3 |
| **Comb(A*B)** | 29 | 1.866396 | 0.064358 | 48.86714 | 1.63 |
| **Error/Res** | 107 | 0.14092 | 0.001317 | | |
| **LSD** | 0.045445 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.49:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein concentrate

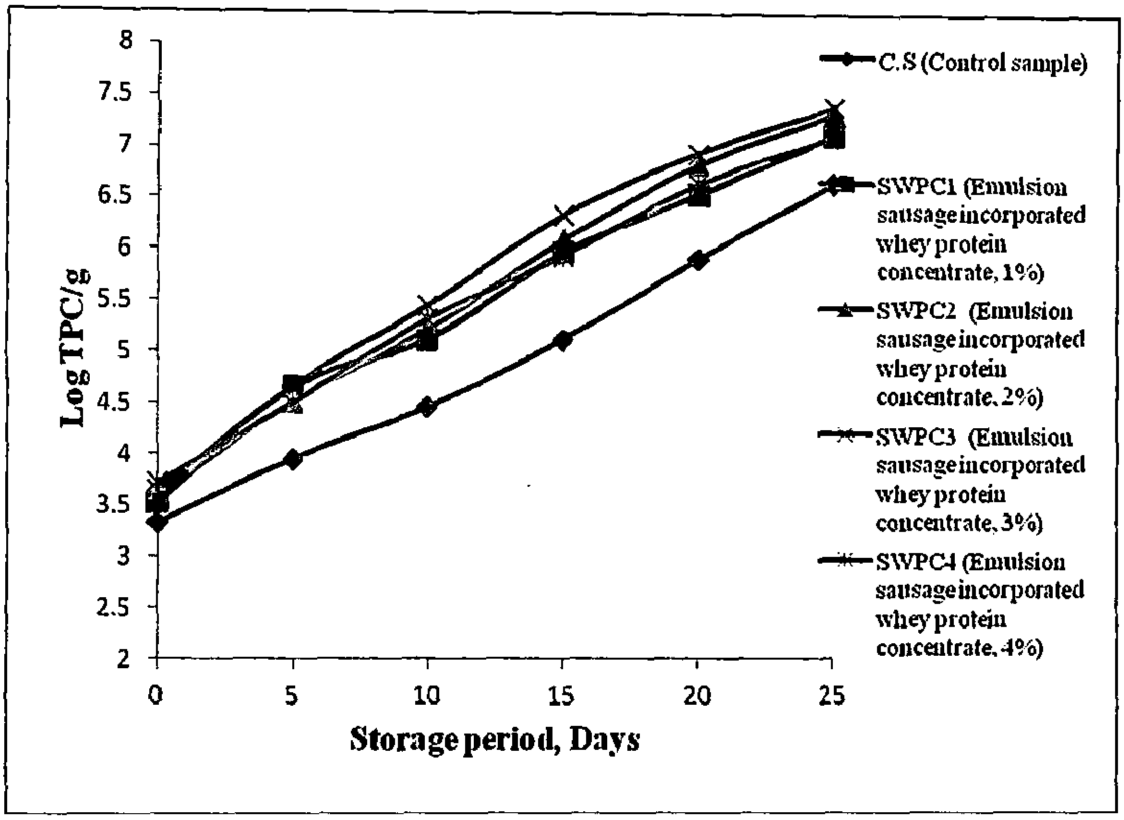



**Fig 4.50:** Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

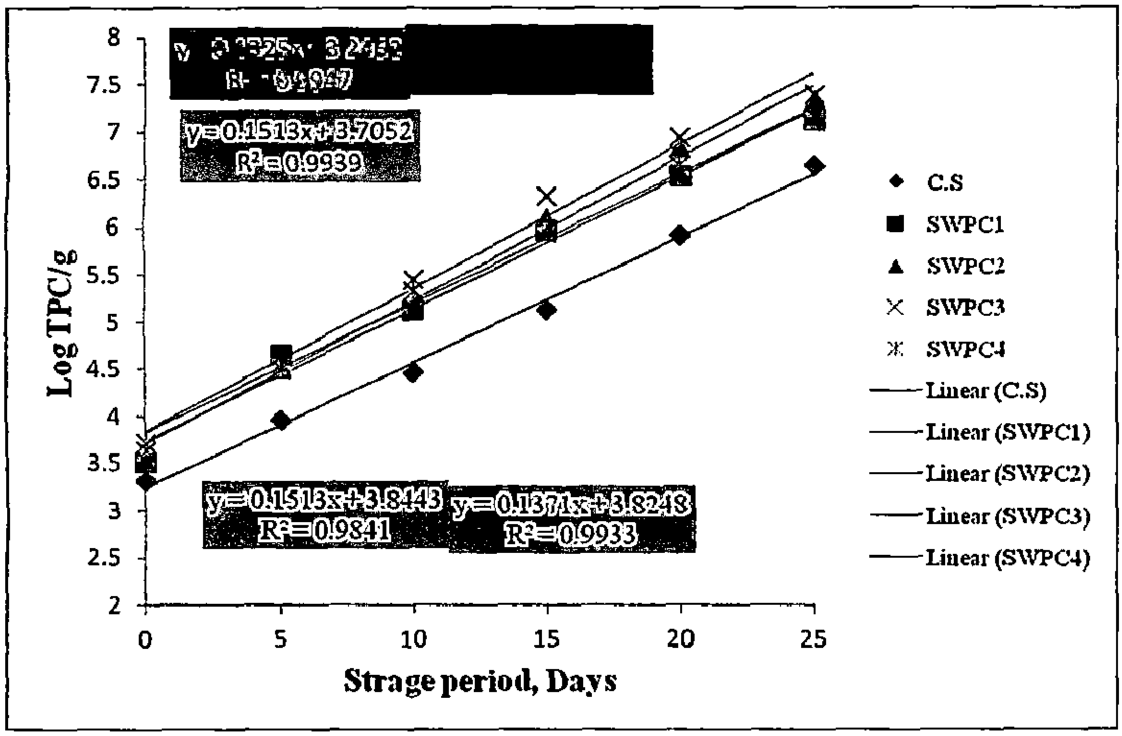


**Table 4.64:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | TPC (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 3.32± 0.017a | 3.95± 0.032 | 4.46± 0.013 | 5.12± 0.016 | 5.91± 0.038 | 6.65± 0.027e |
| **$Swpi_1$** | 3.69± 0.013b | 4.13± 0.020 | 4.85± 0.027 | 5.64± 0.031 | 6.15± 0.033 | 6.90± 0.038f |
| **$Swpi_2$** | 3.98± 0.012c | 4.94± 0.038 | 5.42± 0.020 | 5.86± 0.032 | 6.45± 0.032 | 7.12± 0.043g |
| **$Swpi_3$** | 3.85± 0.037d | 4.42± 0.018 | 5.33± 0.020 | 5.95± 0.035 | 6.61± 0.032 | 7.23± 0.014h |
| **$Swpi_4$** | 3.80± 0.055d | 4.33± 0.031 | 4.95± 0.025 | 5.44± 0.029 | 5.95± 0.035 | 6.74± 0.026i |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.65:** ANOVA of total plate count of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 188.5237 | 1.26526 | | |
| **Replicate** | 4 | 0.004393 | 0.001098 | 1.077785 | 2.46 |
| **FA** | 4 | 10.59585 | 2.648962 | 2599.403 | 2.46 |
| **FB** | 5 | 175.9224 | 35.18449 | 34526.23 | 2.3 |
| **Comb(A*B)** | 29 | 1.896409 | 0.065393 | 64.17 | 1.63 |
| **Error/Res** | 107 | 0.10904 | 0.001019 | | |
| **LSD** | 0.039976 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.51:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein isolate

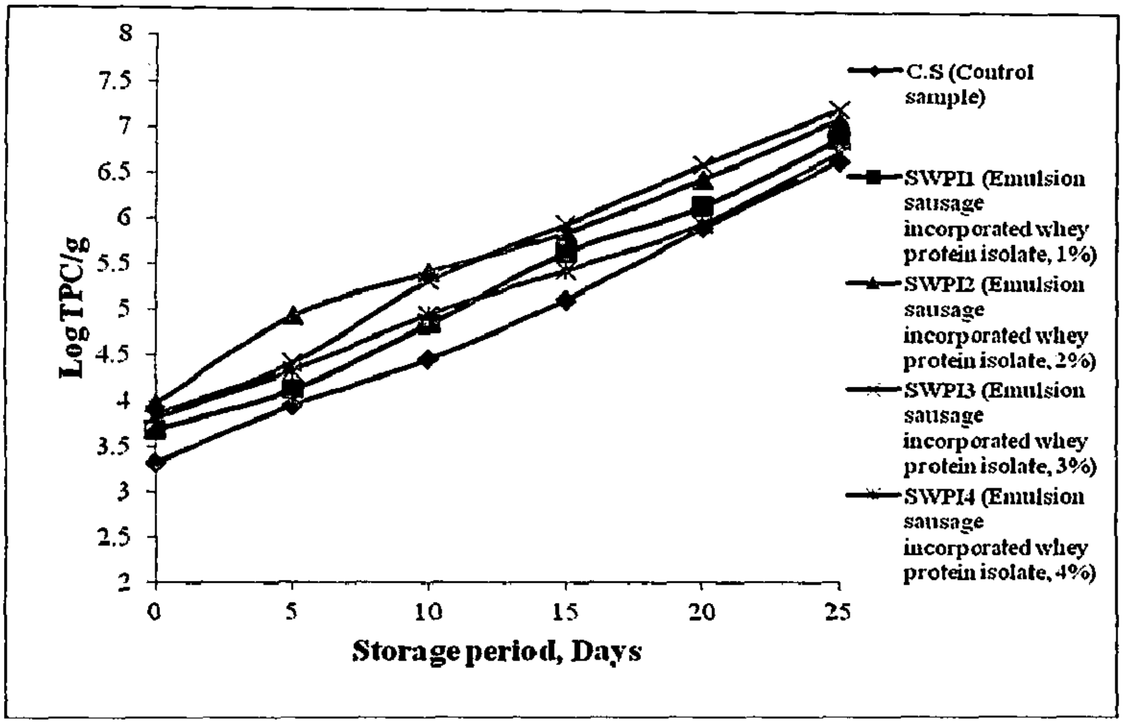



**Fig 4.52:** Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

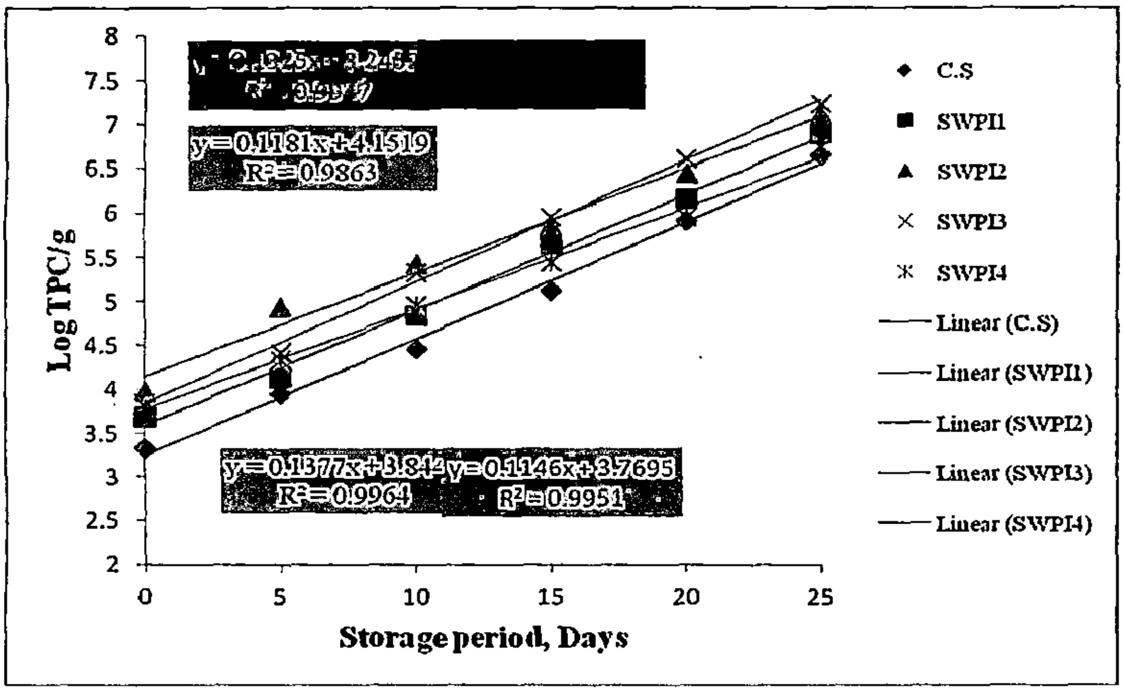


**Table 4.66:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | TPC (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 3.32± 0.017a | 3.95± 0.032 | 4.46± 0.013 | 5.12± 0.016 | 5.91± 0.038 | 6.65± 0.027d |
| $Swpp_1$ | 3.70± 0.082b | 4.45± 0.060 | 5.48± 0.067 | 5.92± 0.048 | 6.42± 0.035 | 7.14± 0.031e |
| $Swpp_2$ | 3.93± 0.032c | 4.55± 0.030 | 5.45± 0.031 | 6.08± 0.041 | 6.85± 0.031 | 7.21± 0.040f |
| $Swpp_3$ | 3.92± 0.030c | 4.55± 0.033 | 4.94± 0.035 | 5.49± 0.049 | 6.27± 0.054 | 6.86± 0.037g |
| $Swpp_4$ | 3.92± 0.048c | 4.45± 0.020 | 4.89± 0.050 | 5.36± 0.033 | 6.10± 0.064 | 6.76± 0.035h |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.67:** ANOVA of total plate count of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 187.6522 | 1.259411 | | |
| Replicate | 4 | 0.012613 | 0.003153 | 1.551723 | 2.46 |
| FA | 4 | 10.38035 | 2.595088 | 1277.016 | 2.46 |
| FB | 5 | 174.1138 | 34.82277 | 17135.93 | 2.3 |
| Comb(A*B) | 29 | 2.940567 | 0.101399 | 49.89734 | 1.63 |
| Error/Res | 107 | 0.21744 | 0.002032 | | |
| LSD | 0.056451 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.53:** Effect of refrigerated storage (0°C) on total plate count of emulsion sausages incorporated with different levels of whey protein powder

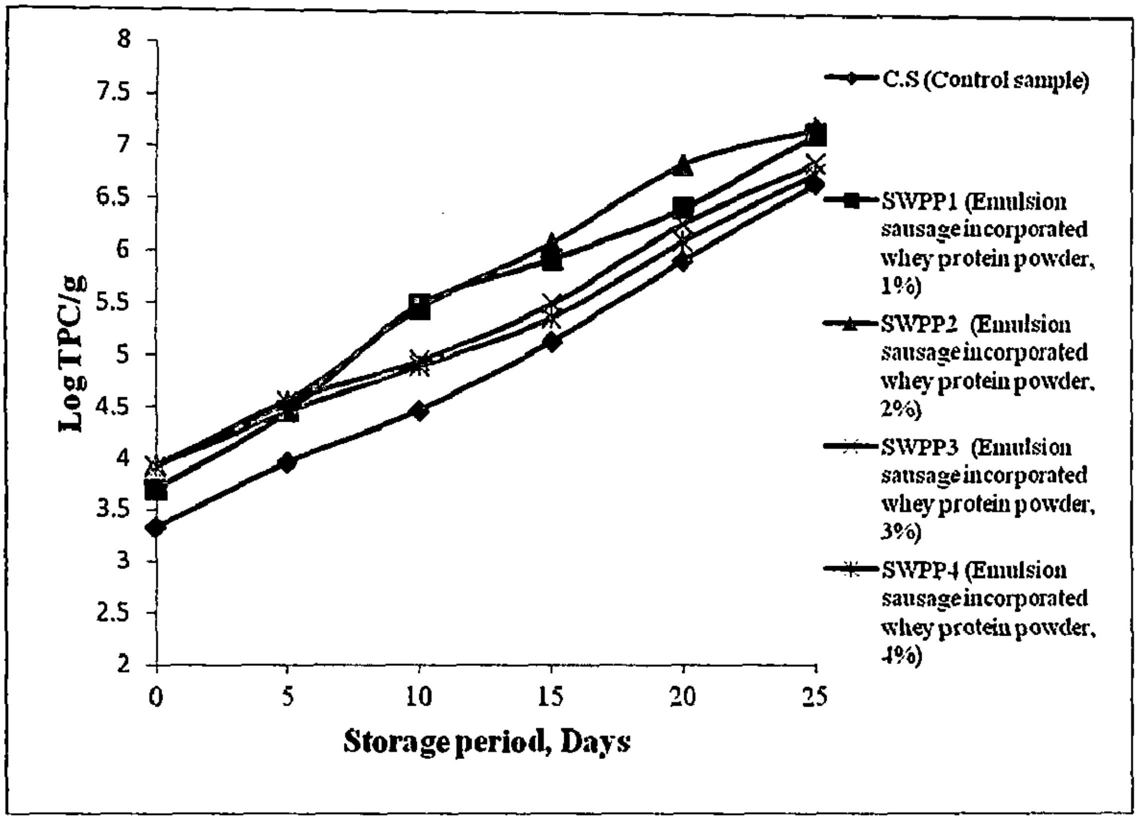
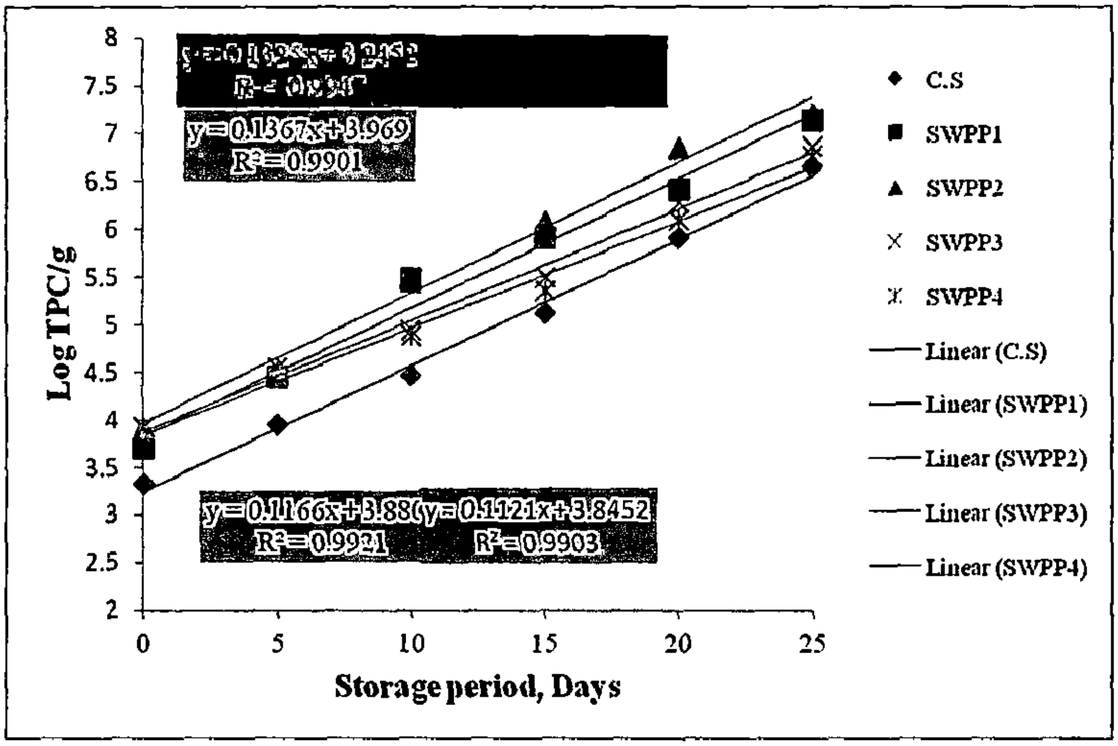



**Fig 4.54:** Regression analysis of total plate count of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.3.2 Yeast and Mold count

The result of yeast and mold counts of sausages samples expressed as log cfu/g has been presented in Tables 4.68, 4.70 and 4.72. Yeast and mold count were not affected in samples till 5 days of refrigerated storage at 0°C. A very low count was observed at 10th day of storage. The results were agreement with Kala *et al.*, (2007) who advocated that yeast and mold counts were observed on 9th and 12th days during refrigerated storage for chicken emulsion patties. However, countable colonies were noted in sausage samples on 25th day of storage in three treatments of whey protein products. The results of yeast and mold count of emulsion sausages incorporated with whey protein concentrate, isolate and powder during refrigerated storage have been plotted in Figure 4.55, 4.57 and 4.59 respectively. Refrigerated storage significantly ($p<0.05$) increased the yeast and mold count of emulsion sausages. The yeast and mold count was found to be between 3.34 - 3.86 log cfu/g, 3.45 - 3.57 log cfu/g and 3.58 - 3.95 log cfu/g for emulsion sausages incorporated with whey protein concentrate, isolate and powder in varied levels (1, 2, 3 and 4 %) respectively. The ANOVA tests indicated that the different levels of whey protein concentrate isolate and powder significantly ($p<0.05$) increased yeast and mold count of emulsion sausages (Table 4.69, 4.71 and 4.73). After 25 days of refrigerated storage (0°C) yeast and mold count of all samples was found to be less than 4 log cfu/g. This particular value of yeast and mold count defined the spoilage condition. When log cfu/g of yeast and mold count increased to 4.0, spoilage of food samples starts (CQIASA, 2003). Casaburi *et al.*, (2007) observed that the growth of yeast and mold in Italian style sausages were controlled during storage after inoculation with Lab starter cultures. They concluded that it could be due to the antagonistic activities of the latter. Erkmen (2008) reported similar observation of Turkish sausage after inclusion with LAB strains as protective cultures. Likewise, Olaoye and Onilude (2010) noted a reduction in the yeast and moulds counts in fresh beef inoculated with LAB starters.

Figures 4.56, 4.58 and 4.60 show the regression analysis of yeast and mold count of emulsion sausages during refrigerated storage at 0°C prepared with different levels of (1, 2, 3 and 4%) of whey protein concentrate, isolate and powder respectively. The equations of the regression and correlation coefficient ($R^2$) have been shown in the regression graph. The positive sign in the coefficients of x explains that there was constant increase yeast and mold count during refrigeration storage.

Values of $R^2$ were very close to 1. Thus, the graph may be approximated to a straight line and linear relation well fits between storage period and yeast and mold count.

**Table 4.68:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Y&M (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | NDa | TFTC | 1.74± 0.041 | 2.08± 0.028 | 2.61± 0.079 | 3.23± 0.050b |
| **$Swpc_1$** | NDa | TFTC | 1.85± 0.038 | 2.26± 0.058 | 2.94± 0.044 | 3.34± 0.034c |
| **$Swpc_2$** | NDa | TFTC | 1.84± 0.028 | 2.22± 0.043 | 2.96± 0.029 | 3.45± 0.031d |
| **$Swpc_3$** | NDa | TFTC | 2.15± 0.041 | 2.43± 0.037 | 2.93± 0.024 | 3.86± 0.031e |
| **$Swpc_4$** | NDa | TFTC | 2.19± 0.051 | 2.40± 0.070 | 2.90± 0.042 | 3.82± 0.052e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%), ND = Not detected, TFTC = To few to count

**Table 4.69:** ANOVA of yeast and mold count of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 276.6585 | 1.856768 | | |
| **Replicate** | 4 | 0.006947 | 0.001737 | 1.081374 | 2.46 |
| **FA** | 4 | 1.680673 | 0.420168 | 261.6272 | 2.46 |
| **FB** | 5 | 273.2565 | 54.6513 | 34029.85 | 2.3 |
| **Comb(A*B)** | 29 | 1.549463 | 0.05343 | 33.26922 | 1.63 |
| **Error/Res** | 107 | 0.17184 | 0.001606 | | |
| **LSD** | 0.050184 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.55:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein concentrate

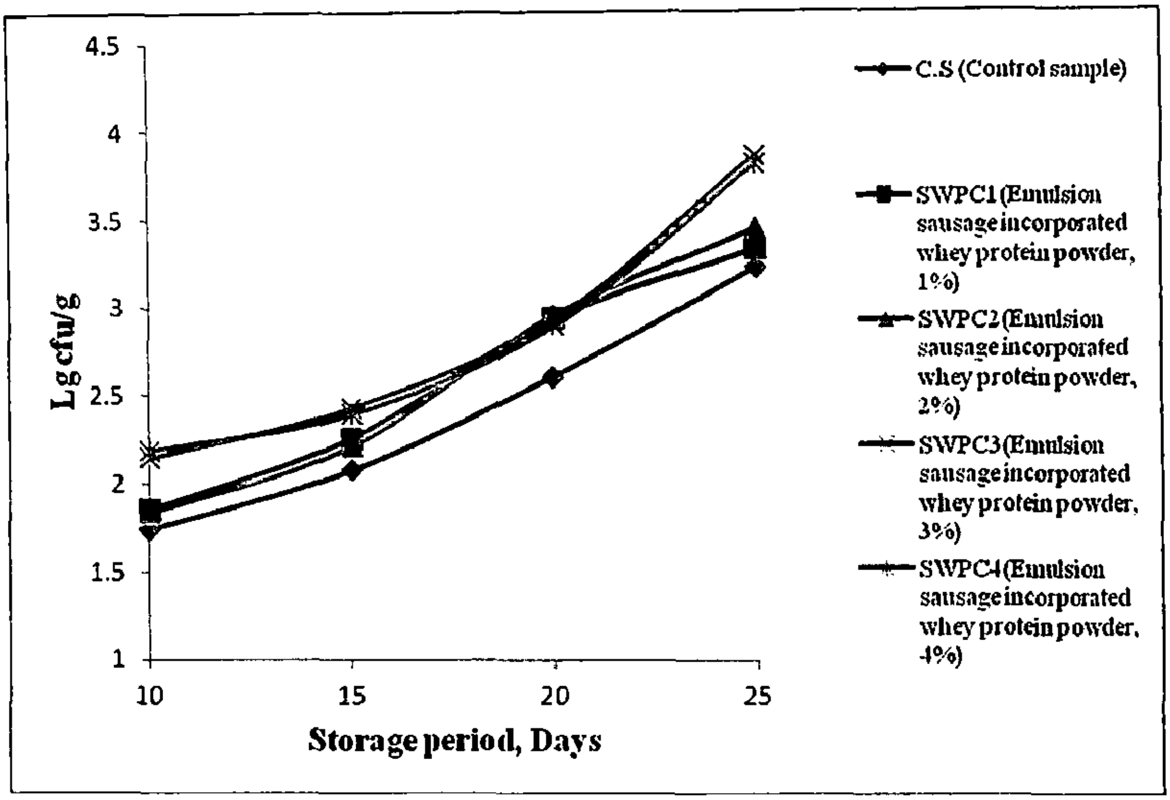



**Fig 4.56:** Regression analysis of yeast and mold count of emulsion sausages incorporated with whey protein concentrate during refrigerated storage (0°C)

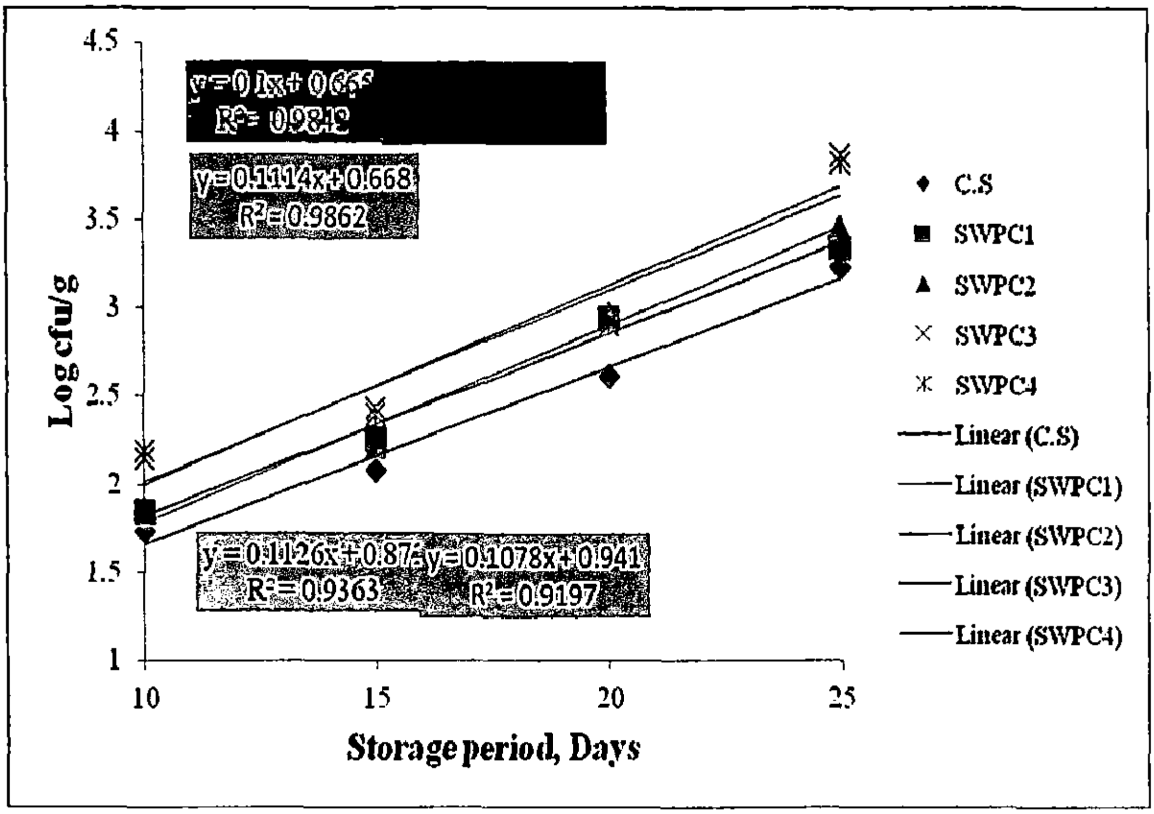


**Table 4.70:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Y&M (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | NDa | TFTC | 1.74± 0.041 | 2.08± 0.028 | 2.61± 0.079 | 3.23± 0.050b |
| $Swpi_1$ | NDa | TFTC | 2.25± 0.049 | 2.84± 0.029 | 3.20± 0.045 | 3.62± 0.042c |
| $Swpi_2$ | NDa | TFTC | 2.18± 0.043 | 2.35± 0.035 | 3.23± 0.058 | 3.55± 0.055d |
| $Swpi_3$ | NDa | TFTC | 2.33± 0.020 | 2.41± 0.021 | 3.12± 0.035 | 3.45± 0.051e |
| $Swpi_4$ | NDa | TFTC | 2.24± 0.038 | 2.53± 0.054 | 3.32± 0.036 | 3.57± 0.053d |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%), ND = Not detected, TFTC = To few to count

**Table 4.71:** ANOVA of yeast and mold count of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 293.4673 | 1.969579 | | |
| Replicate | 4 | 0.007143 | 0.001786 | 1.145207 | 2.46 |
| FA | 4 | 2.570209 | 0.642552 | 412.09 | 2.46 |
| FB | 5 | 288.6862 | 57.73725 | 37028.8 | 2.3 |
| Comb(A*B) | 29 | 2.044039 | 0.070484 | 45.20378 | 1.63 |
| Error/Res | 107 | 0.16684 | 0.001559 | | |
| LSD | 0.049449 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.57:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein isolate

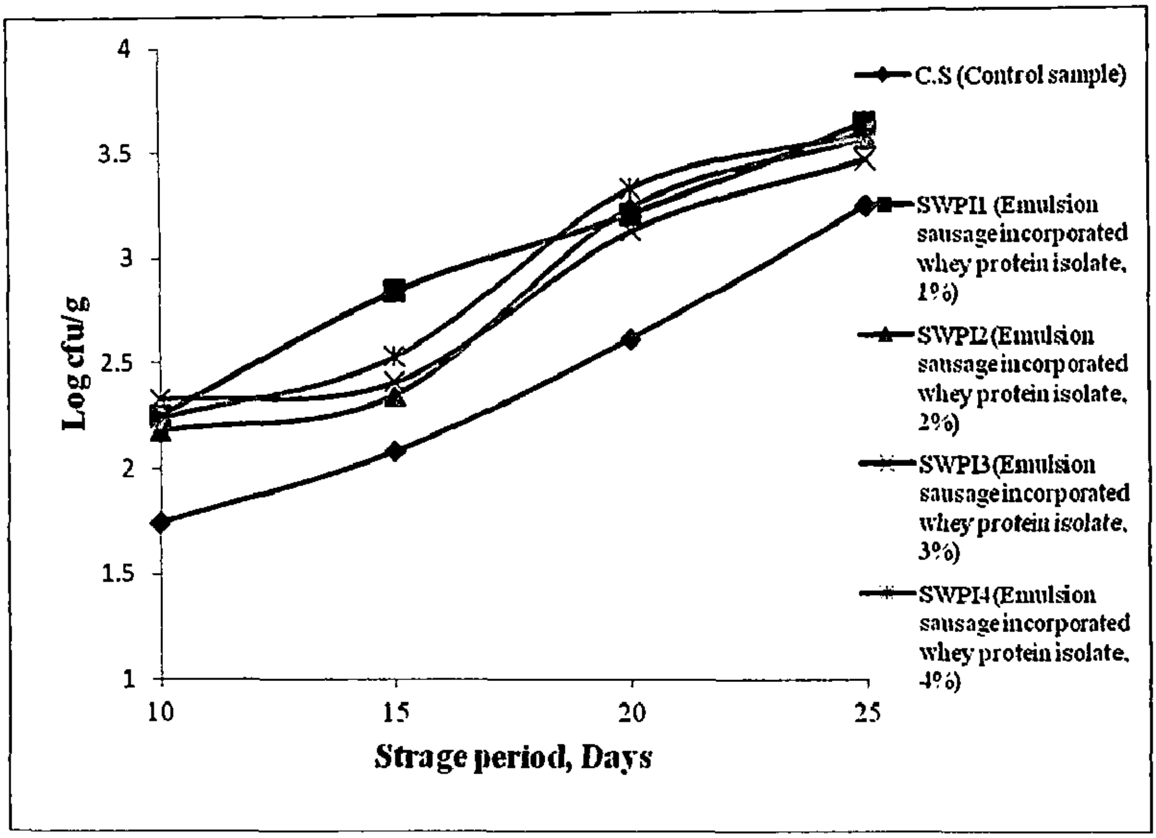



**Fig 4.58:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein powder

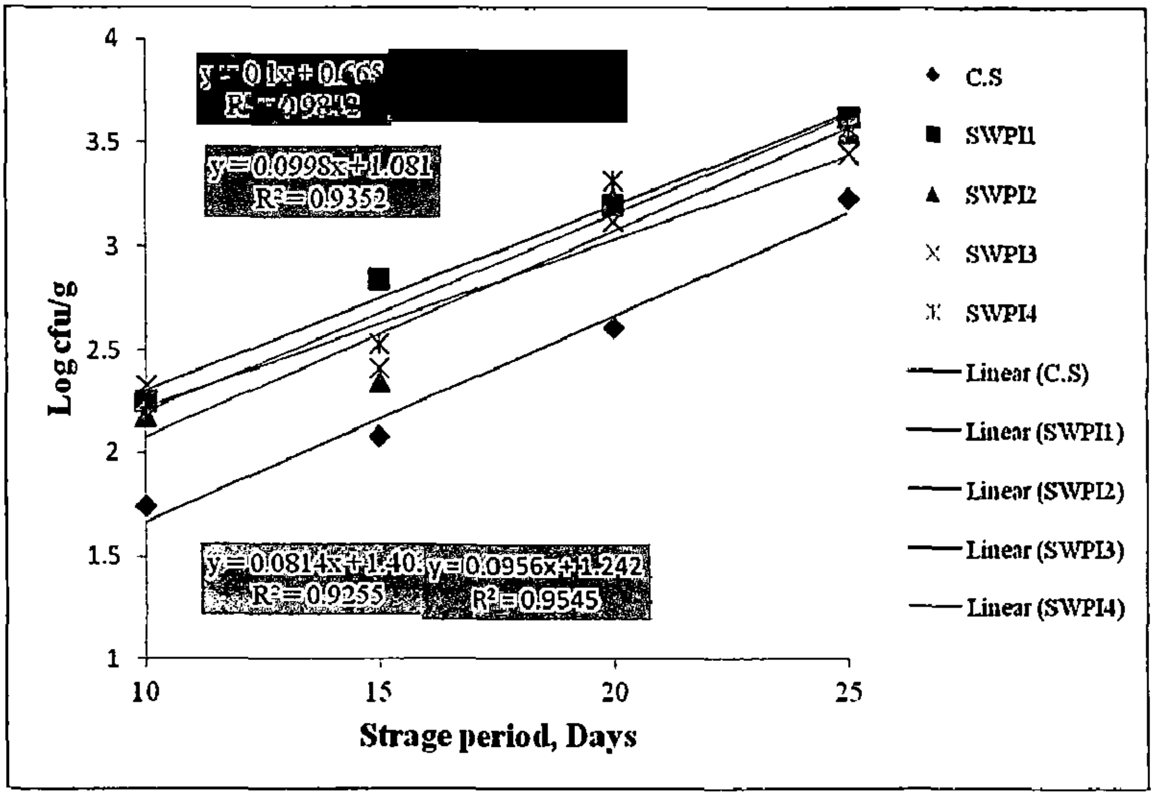


**Table 4.72:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Y&M (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | NDa | TFTC | 1.74± 0.041 | 2.08± 0.028 | 2.61± 0.079 | 3.23± 0.050b |
| $Swpp_1$ | NDa | TFTC | 1.93± 0.041 | 2.14± 0.037 | 2.86± 0.074 | 3.58± 0.049c |
| $Swpp_2$ | NDa | TFTC | 2.24± 0.025 | 2.76± 0.085 | 3.23± 0.041 | 3.89± 0.049d |
| $Swpp_3$ | NDa | TFTC | 2.11± 0.051 | 2.65± 0.057 | 3.15± 0.055 | 3.79± 0.049e |
| $Swpp_4$ | NDa | TFTC | 2.34± 0.028 | 2.73± 0.079 | 3.30± 0.061 | 3.95± 0.088f |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%), ND = Not detected, TFTC = To few to count

**Table 4.73:** ANOVA of yeast and mold count of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 308.8569 | 2.072865 | | |
| **Replicate** | 4 | 0.006187 | 0.001547 | 0.663353 | 2.46 |
| **FA** | 4 | 4.26654 | 1.066635 | 457.4713 | 2.46 |
| **FB** | 5 | 301.929 | 60.3858 | 25898.99 | 2.3 |
| **Comb(A*B)** | 29 | 2.411844 | 0.083167 | 35.66968 | 1.63 |
| **Error/Res** | 107 | 0.24948 | 0.002332 | | |
| **LSD** | 0.060467 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.59:** Effect of refrigerated storage (0°C) on yeast and mold count of emulsion sausages incorporated with different levels of whey protein powder

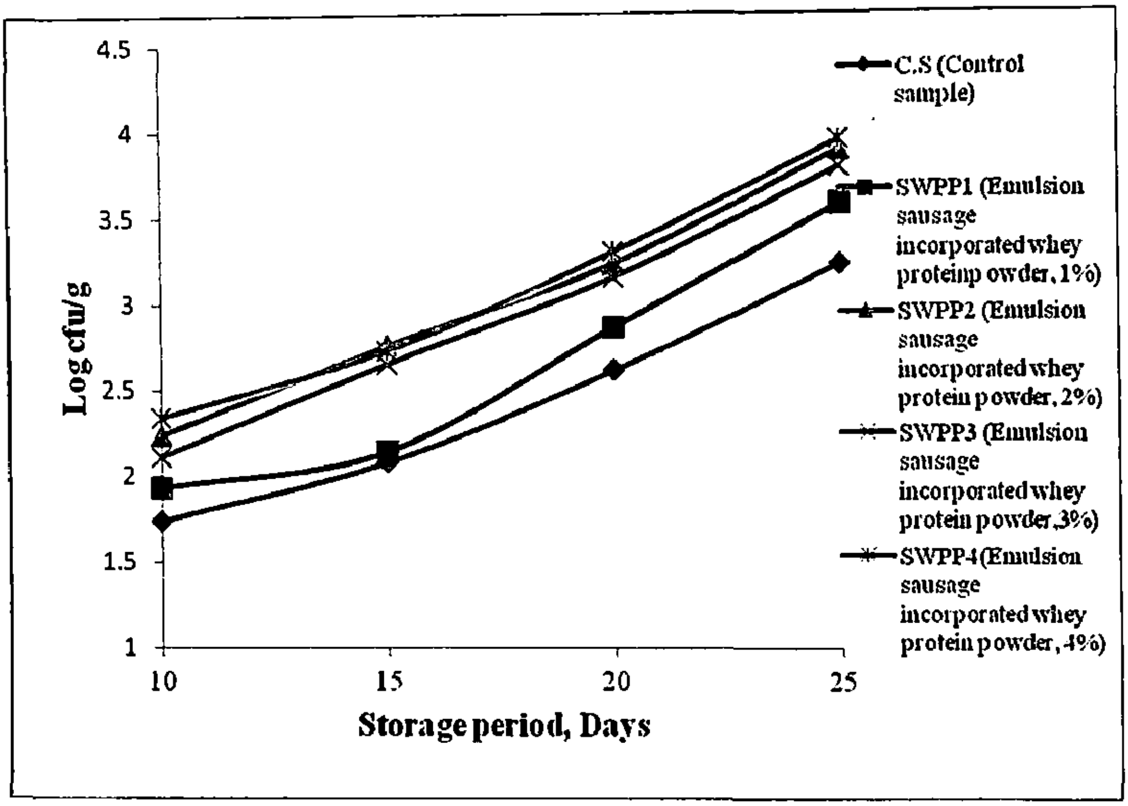
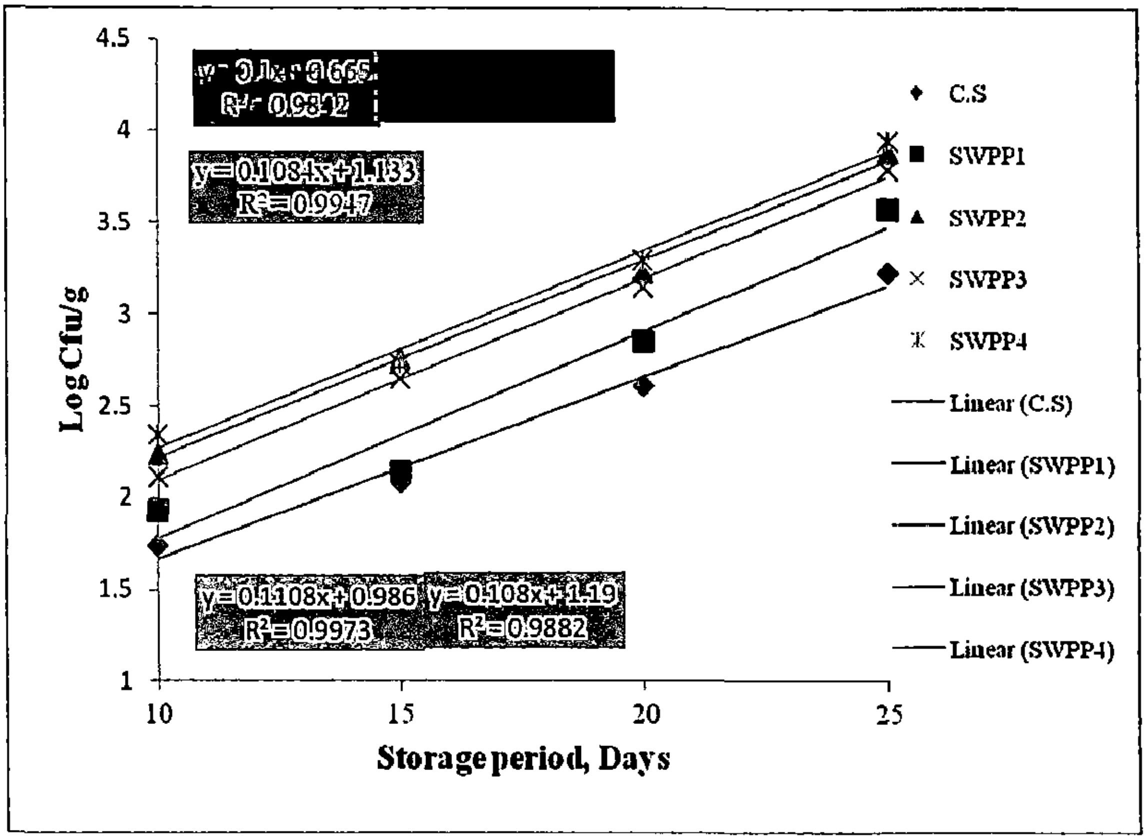



**Fig 4.60:** Regression analysis of yeast and mold count of emulsion sausages incorporated with whey protein powder during refrigerated storage (0°C)



### 4.2.3.3 Coliform count

Coliform count of emulsion sausages produced with different whey protein product namely whey protein concentrate, isolate and powder were enumerated and it was found that there were no sign of coliform bacteria on plates containing MacConkey agar till 20$^{th}$ day of storage (0°C) the incorporation of whey protein concentrate, isolate and powder in four levels (1, 2, 3 and 4%) were selected for study. However, coliform count of sausages samples were found to be in the range of 2.06-2.34 log cfu/g, 2.15-2.28 log cfu/g and 2.08-2.22 log cfu/g in emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively after 15 days of storage (Tables 4.74, 4.76 and 4.78). The results are in agreement with Kala *et al.*, (2007) who found that the coliforms were totally undetected from any of the emulsions chicken patties during 12 day of storage. The refrigerated storage significantly ($p<0.05$) increased the coliform count (ANOVA Table 7.75, 4.77 and 4.79). In final stage of storage, coliform count was found to be in the range of 2.16-2.54, 2.30-2.41and 2.30-2.43 log cfu/g. The results of coliform count of emulsion sausages incorporated with whey protein concentrate, isolate and powder during refrigerated storage have been plotted in Figure 4.61, 4.62 and 4.53 respectively. Reduction in counts of *Enterabacteriaceae* and *Staphylococcus* in meat has been reported by (Gomolka- Pawlicka *et al.*, 2004; Koban and Kaya 2006; Olaoye and Onilude, 2010). Usually, the presence of total coliform in food indicates improper heat treatment or post processing contamination. Coliform were not usually pathogenic. They also indicate inadequate sanitation and disinfection of appliance (CQIASA, 2003).

**Table 4.74:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Coliform count (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | NDa | ND | ND | ND | 2.06±0.022 | 2.16±0.022b |
| Swpc$_1$ | NDa | ND | ND | ND | 2.30±0.028 | 2.54±0.031c |
| Swpc$_2$ | NDa | ND | ND | ND | 2.21±0.036 | 2.35±0.022d |
| Swpc$_3$ | NDa | ND | ND | ND | 2.34±0.025 | 2.54±0.025c |
| Swpc$_4$ | NDa | ND | ND | ND | 2.11±0.022 | 2.25±0.036e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpc$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%), ND = Not detected

**Table 4.75:** ANOVA of coliform count of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 176.2802 | 1.183088 | | |
| Replicate | 4 | 0.001211 | 0.000303 | 1.024852 | 2.46 |
| FA | 4 | 0.277957 | 0.069489 | 235.2962 | 2.46 |
| FB | 5 | 175.3839 | 35.07678 | 118772.6 | 2.3 |
| Comb(A*B) | 29 | 0.586715 | 0.020232 | 68.50553 | 1.63 |
| Error/Res | 107 | 0.0316 | 0.000295 | | |
| LSD | 0.02152 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.61:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein concentrate

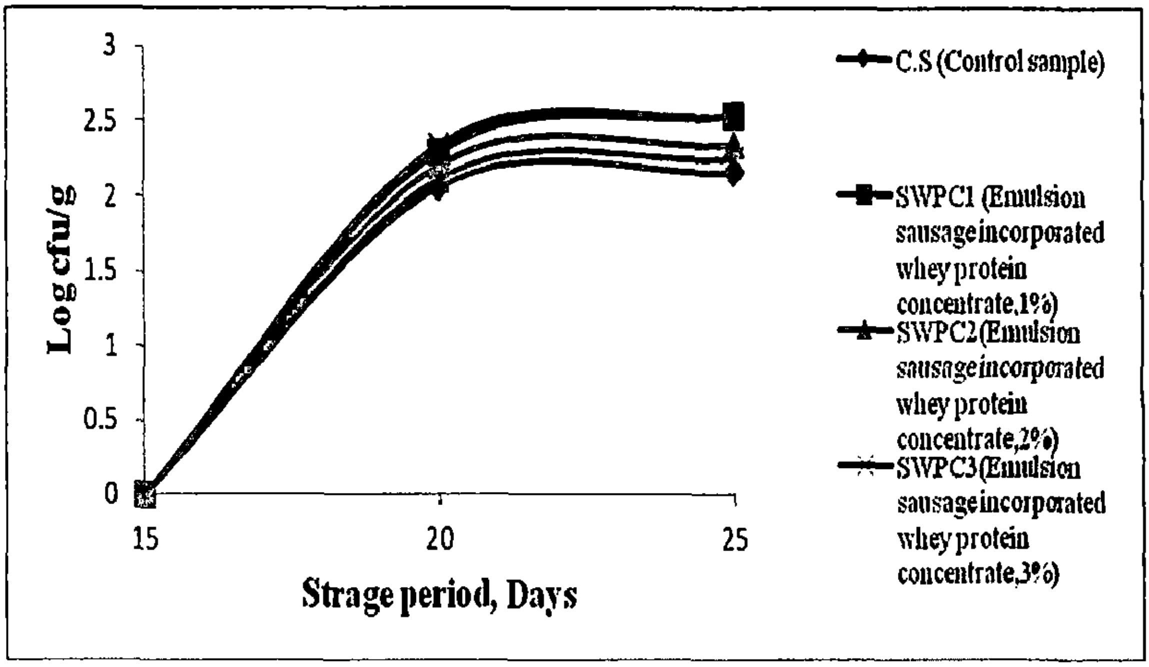


**Table 4.76:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Coliform count (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | NDa | ND | ND | ND | 2.06±0.022 | 2.16±0.022b |
| $Swpi_1$ | NDa | ND | ND | ND | 2.28±0.068 | 2.35±0.033c |
| $Swpi_2$ | NDa | ND | ND | ND | 2.22±0.087 | 2.30±0.040c |
| $Swpi_3$ | NDa | ND | ND | ND | 2.15±0.032 | 2.38±0.049c |
| $Swpi_4$ | NDa | ND | ND | ND | 2.19±0.061 | 2.41±0.056d |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%), ND = Not detected

**Table 4.77:** ANOVA of coliform count of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 170.2412 | 1.142558 | | |
| Replicate | 4 | 0.003071 | 0.000768 | 0.767667 | 2.46 |
| FA | 4 | 0.085471 | 0.021368 | 21.36767 | 2.46 |
| FB | 5 | 169.813 | 33.9626 | 33962.6 | 2.3 |
| Comb(A*B) | 29 | 0.235721 | 0.008128 | 8.128322 | 1.63 |
| Error/Res | 107 | 0.107 | 0.001 | | |
| LSD | 0.0396 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.62:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein isolate

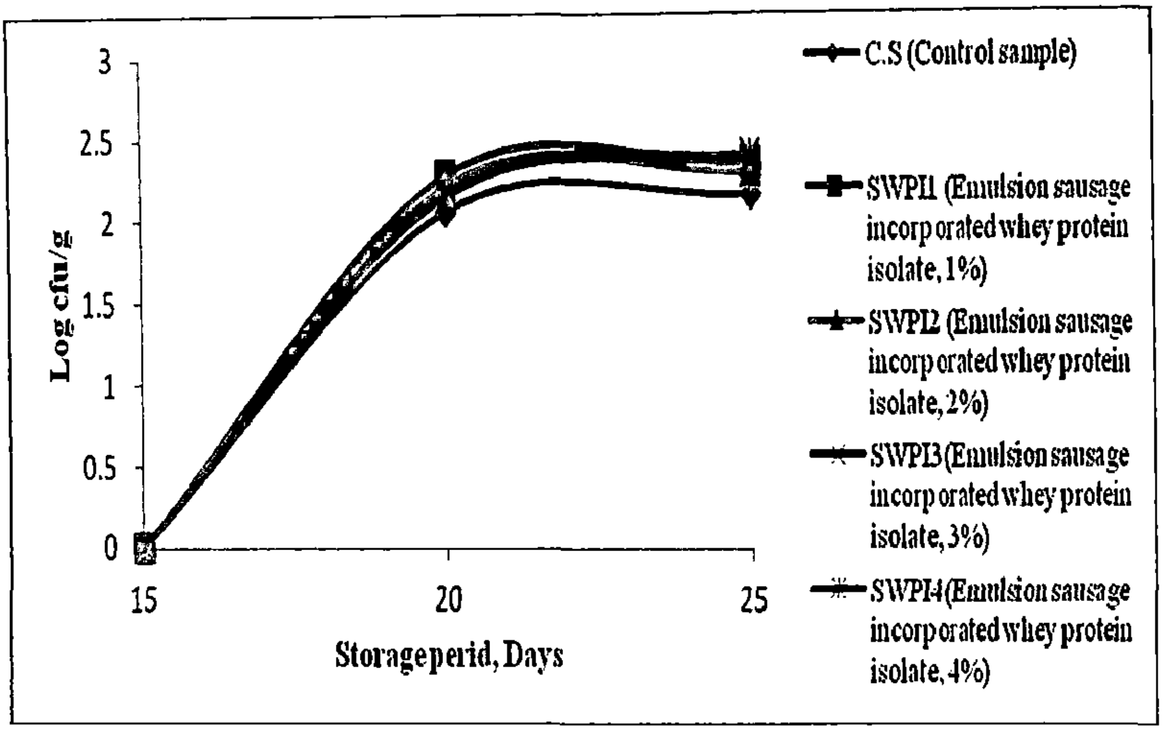



**Table 4.78:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Coliform count (Log cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | NDa | ND | ND | ND | 2.06±0.022 | 2.16±0.022b |
| $Swpp_1$ | NDa | ND | ND | ND | 2.15±0.040 | 2.30±0.034c |
| $Swpp_2$ | NDa | ND | ND | ND | 2.11±0.043 | 2.34±0.044c |
| $Swpp_3$ | NDa | ND | ND | ND | 2.08±0.048 | 2.40±0.060d |
| $Swpp_4$ | NDa | ND | ND | ND | 2.22±0.026 | 2.43±0.065d |

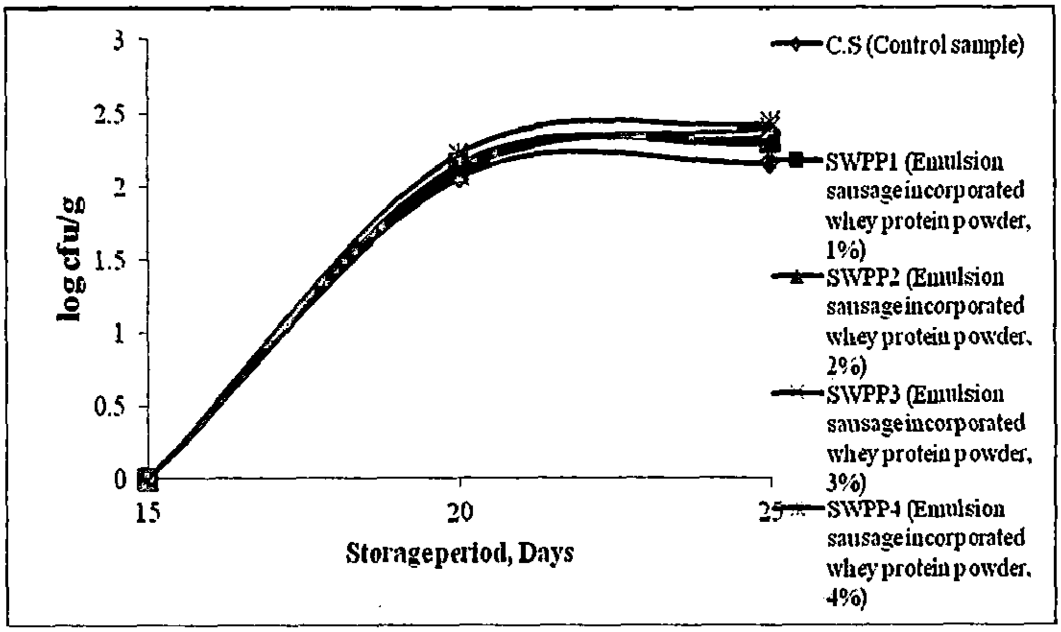
Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%), ND = Not detected

**Table 4.79:** ANOVA of coliform count of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 166.5941 | 1.118081 | | |
| **Replicate** | 4 | 0.000631 | 0.000158 | 0.226508 | 2.46 |
| **FA** | 4 | 0.072551 | 0.018138 | 26.05707 | 2.46 |
| **FB** | 5 | 166.2282 | 33.24563 | 47761.58 | 2.3 |
| **Comb(A*B)** | 29 | 0.218873 | 0.007547 | 10.84274 | 1.63 |
| **Error/Res** | 107 | 0.07448 | 0.000696 | | |
| **LSD** | 0.033039 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.63:** Effect of refrigerated storage (0°C) on coliform count of emulsion sausages incorporated with different levels of whey protein powder




### 4.2.3.4 *Salmonella shigella* count

*Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C for 25 days. These findings further support the idea of Kala *et al.,* (2007) Salmonella could not be detected from the emulsion patties samples during various periods of storage.

### 4.2.4 Sensory characteristics of buffalo meat emulsion sausage during refrigerated storage 0°C

#### 4.2.4.1 Colour

The emulsion sausages had bright red colour after cooking. All the fresh emulsion sausages incorporated with whey protein concentrate, isolate and powder at different levels of (1, 2, 3 and 4%) had score values of colour between 8-9 (Table 4.80, 4.82 and 4.84). It represented condition between liked very much and liked extremely. Different levels of whey protein products significantly ($p<0.05$) increased the colour score values of fresh emulsion sausages as compared to control sample. The colour score values of all samples during storage were found to be decreasing. The ANOVA (Table 4.81, 4.83 and 4.85) results indicated that refrigerated storage significantly ($p<0.05$) decreased the colour score values of emulsion sausage. Figures (4.64, 4.66 and 4.68) represent the decreasing profile of colour score values of emulsion sausages samples during refrigerated storage. The findings of the present study are consistent with Kala *et al.*, (2007), who found the colour score values significantly decreased for chicken emulsion patties with the storage period in days. Texture and colour was found to change when the pH was lowered, as some of the proteins started to coagulate (Ngapo *et al.*, 1996; Barbut, 2005). After 25 days of storage, the highest score for colour was exhibited by sample of emulsion sausage incorporated with whey protein at the levels of 4%. The decline in colour scores of the samples during storage was due to lipid oxidation and subsequent oxidized compounds reacting with amino acids during non enzymatic browning of the product (Choi *et al.*, 2003). Bhaskar *et al.*, (2009) reported that irrespective of type of formulations and type of storage conditions, the mean colour, flavor, juiciness, tenderness and overall acceptability scores of pork sausages decreased significantly ($p<0.01$) with increasing storage period. This reduction in mean colour scores of stored pork sausages might be due to oxidative fading, the reduced flavor scores might be due to the fat oxidation during storage and reduced juiciness and tenderness scores might be due to loss of moisture and fat as storage period progressed. Similar type of results were noticed by Pangas *et al.*, (1999) in fried chicken liver, Rao *et al.*, (1996) in chicken sausages added with whey protein concentrate up to 2.5 percent level. Bhoyar *et al.*, (1998) also observed similar results in restructured chicken steaks extended with 10% textured soy protein.

Figures (4.65, 4.67 and 4.69) represent the linear regression of colour score of sausages samples during refrigerated storage. The equation of regression line and correlation coefficients are shown on the regression graph. The negative sign in the coefficients of x explains that there was constant decrease of colour score values during storage period. The values of $R^2$ for all samples at four levels of whey protein concentrate, isolate and powder were between 0.9292-0.9498, 0.9363-0.9898 and 0.9429-0.9842 respectively. This shows relation between colour score and storage period were almost perfect and the graph may be approximated to a straight line.

**Table 4.80:** Effect of refrigerated storage (0°C) on colour of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Colour score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.7± 0.01a | 7.7± 0.03 | 7.4± 0.05 | 7.1± 0.01 | 7.1± 0.02 | 6.8± 0.04c |
| $Swpc_1$ | 8.7± 0.02ab | 8.1± 0.07 | 7.8± 0.03 | 7.5± 0.05 | 7.5± 0.04 | 7.1± 0.03d |
| $Swpc_2$ | 8.6± 0.01ab | 8.4± 0.04 | 8.1± 0.07 | 7.6± 0.05 | 7.3± 0.03 | 7.1± 0.03d |
| $Swpc_3$ | 8.1± 0.09b | 8.1± 0.08 | 8.0± 0.01 | 7.9± 0.02 | 7.6± 0.03 | 7.2± 0.06d |
| $Swpc_4$ | 8.4± 0.02b | 8.3± 0.02 | 8.1± 0.01 | 8.1± 0.06 | 7.8± 0.04 | 7.6± 0.04e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.81:** ANOVA of colour of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 36.64254 | 0.245923 | | |
| Replicate | 4 | 0.039796 | 0.009949 | 1.154101 | 2.46 |
| FA | 4 | 9.316763 | 2.329191 | 270.1902 | 2.46 |
| FB | 5 | 23.75844 | 4.751688 | 551.2041 | 2.3 |
| Comb(A*B) | 29 | 2.644933 | 0.091205 | 10.57989 | 1.63 |
| Error/Res | 107 | 0.9224 | 0.008621 | | |
| LSD | 0.116269 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.64:** Effect of refrigerated storage (0°C) on colour of emulsion sausages incorporated with different levels of whey protein concentrate

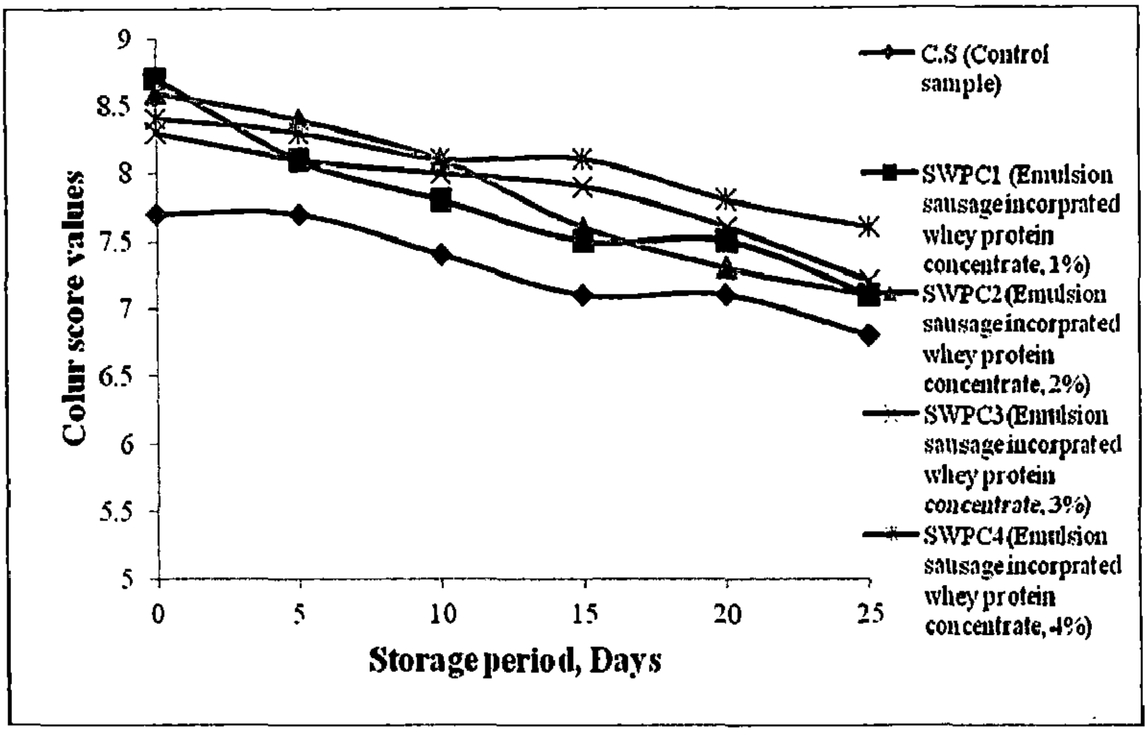



**Fig 4.65:** Regression analysis of colour of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

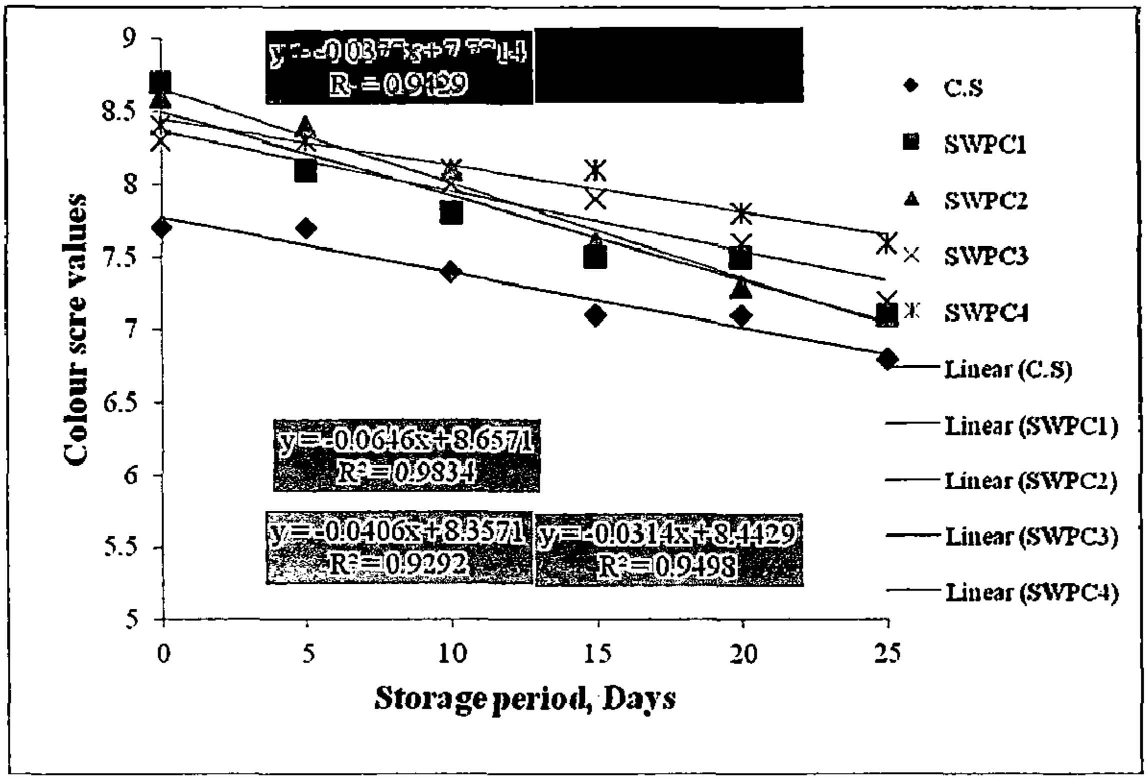


**Table 4.82:** Effect of refrigerated storage (0°C) on color of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Colour score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.7± 0.01a | 7.7± 0.03 | 7.4± 0.05 | 7.1± 0.01 | 7.1± 0.02 | 6.8± 0.04d |
| $Swpi_1$ | 8.0± 0.07b | 7.8± 0.11 | 7.7± 0.08 | 7.3± 0.08 | 7.0± 0.14 | 6.6± 0.11e |
| $Swpi_2$ | 8.6± 0.05c | 8.1± 0.08 | 7.9± 0.19 | 7.7± 0.70 | 7.4± 0.11 | 6.6± 0.22e |
| $Swpi_3$ | 8.7± 0.08c | 8.2± 0.08 | 8.0± 0.05 | 7.6± 0.13 | 7.3± 0.14 | 6.7± 0.19e |
| $Swpi_4$ | 8.4± 0.08c | 8.2± 0.12 | 8.0± 0.16 | 7.6± 0.11 | 7.4± 0.21 | 7.1± 0.56f |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.83:** ANOVA of colour of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 50.69708 | 0.340249 | | |
| Replicate | 4 | 0.15786 | 0.039465 | 1.40706 | 2.46 |
| FA | 4 | 5.709733 | 1.427433 | 50.89279 | 2.46 |
| FB | 5 | 39.23854 | 7.847708 | 279.7971 | 2.3 |
| Comb(A*B) | 29 | 2.747691 | 0.094748 | 3.378083 | 1.63 |
| Error/Res | 107 | 3.00112 | 0.028048 | | |
| LSD | 0.209722 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.66:** Effect of refrigerated storage (0°C) on colour of emulsion sausages incorporated with different levels of whey protein isolate

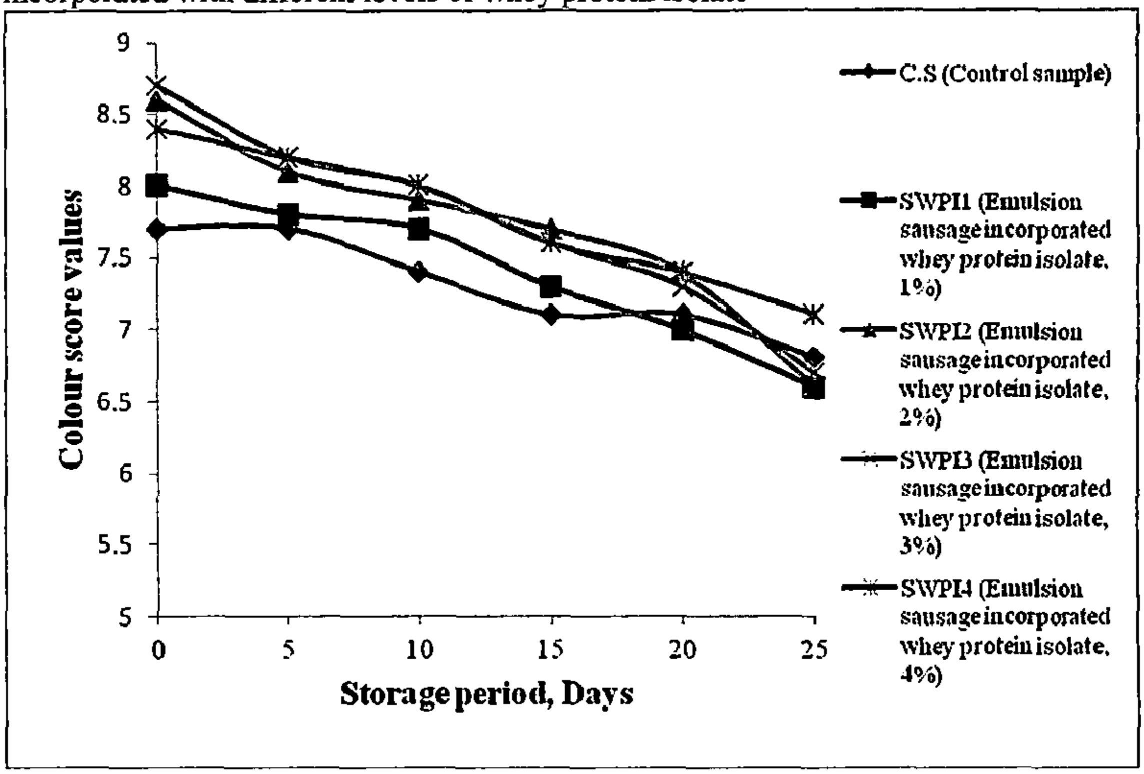



**Fig 4.67:** Regression analysis of colour of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

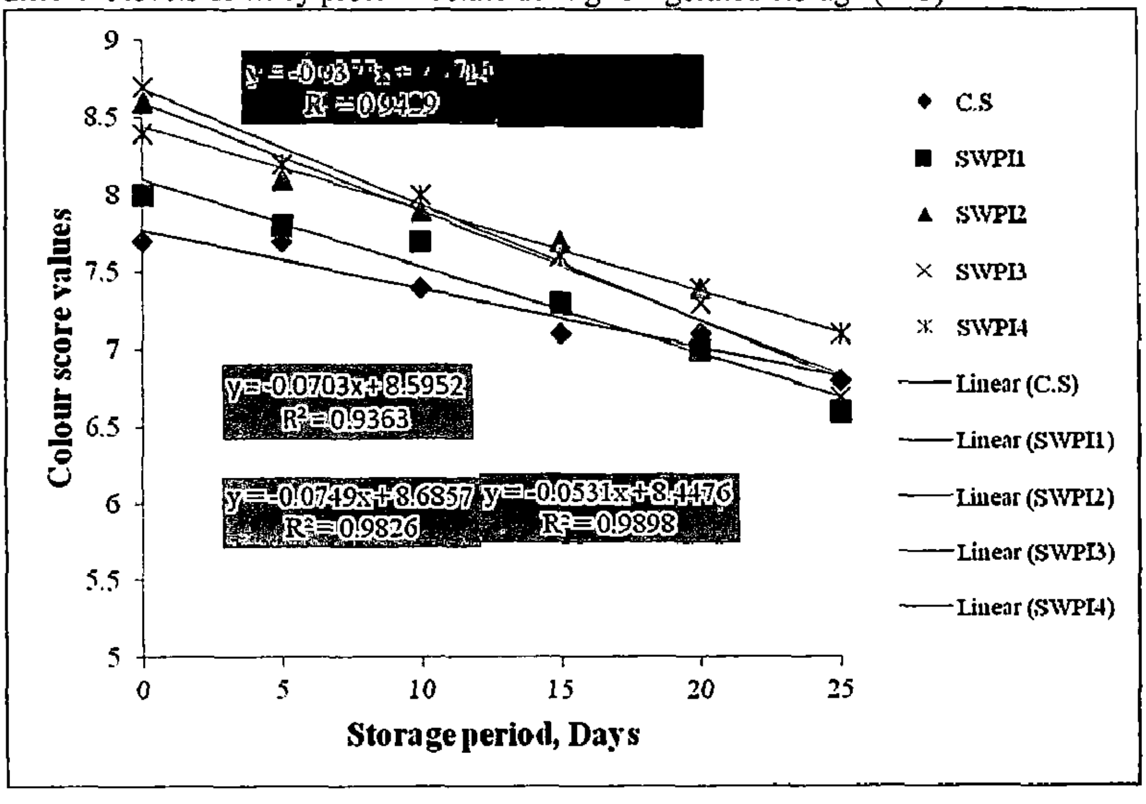


**Table 4.84:** Effect of refrigerated storage (0°C) on color of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Colour score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.7± 0.01a | 7.7± 0.03 | 7.4± 0.05 | 7.1± 0.01 | 7.1± 0.02 | 6.8± 0.04c |
| **$Swpp_1$** | 8.3± 0.02b | 8.1± 0.11 | 7.7± 0.14 | 7.1± 0.19 | 6.9± 0.11 | 6.5± 0.16cd |
| **$Swpp_2$** | 8.3± 0.01b | 8.2± 0.08 | 8.0± 0.18 | 7.3± 0.21 | 7.0± 0.21 | 6.8± 0.18c |
| **$Swpp_3$** | 8.1± 0.01b | 7.9± 0.11 | 7.7± 0.08 | 7.6± 0.10 | 7.3± 0.08 | 6.9± 0.10c |
| **$Swpp_4$** | 7.9± 0.03ac | 7.7± 0.16 | 7.4± 0.32 | 7.0± 0.08 | 6.8± 0.14 | 6.3± 0.15cd |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), C.S = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.85:** ANOVA of colour of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 47.84032 | 0.321076 | | |
| **Replicate** | 4 | 0.071297 | 0.017824 | 0.870583 | 2.46 |
| **FA** | 4 | 4.220751 | 1.055188 | 51.53789 | 2.46 |
| **FB** | 5 | 38.6122 | 7.722439 | 377.1824 | 2.3 |
| **Comb(A*B)** | 29 | 2.816649 | 0.097126 | 4.743858 | 1.63 |
| **Error/Res** | 107 | 2.19072 | 0.020474 | | |
| **LSD** | 0.179183 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.68:** ffect of refrigerated storage (0°C) on colour of emulsion sausages incorporated with different levels of whey protein powder

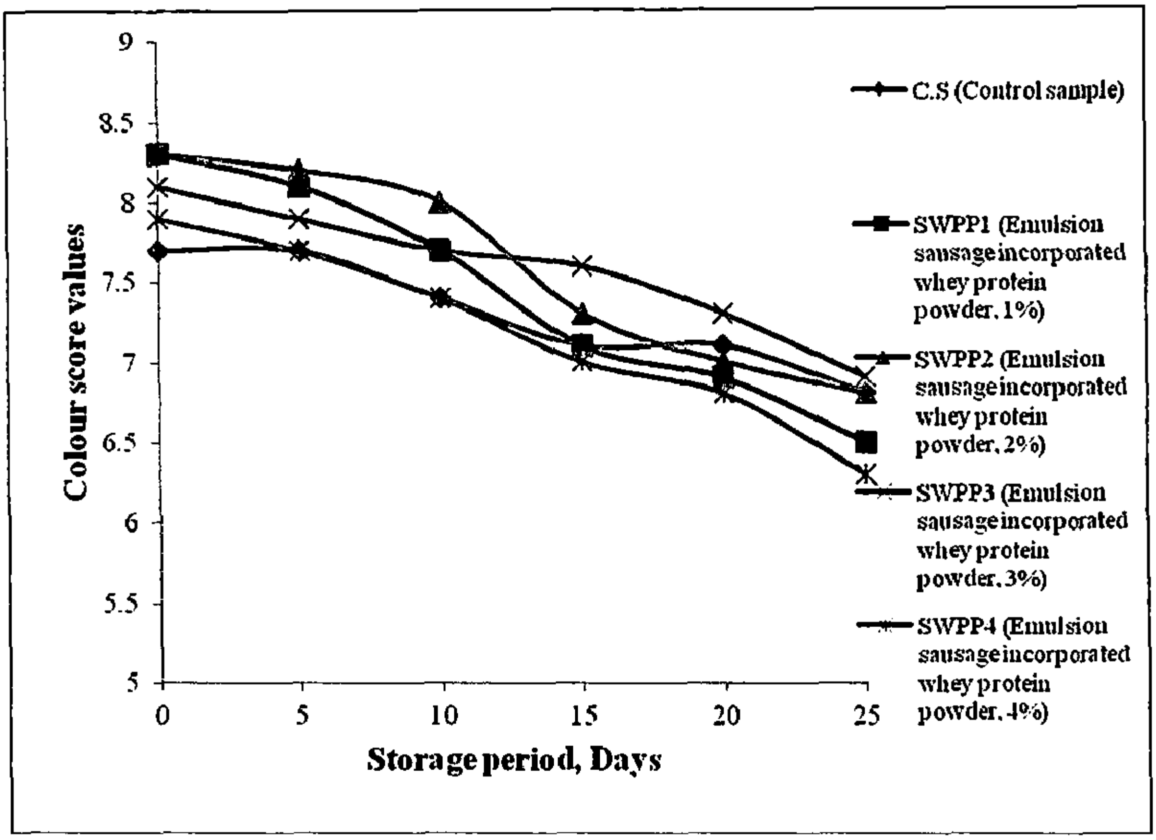
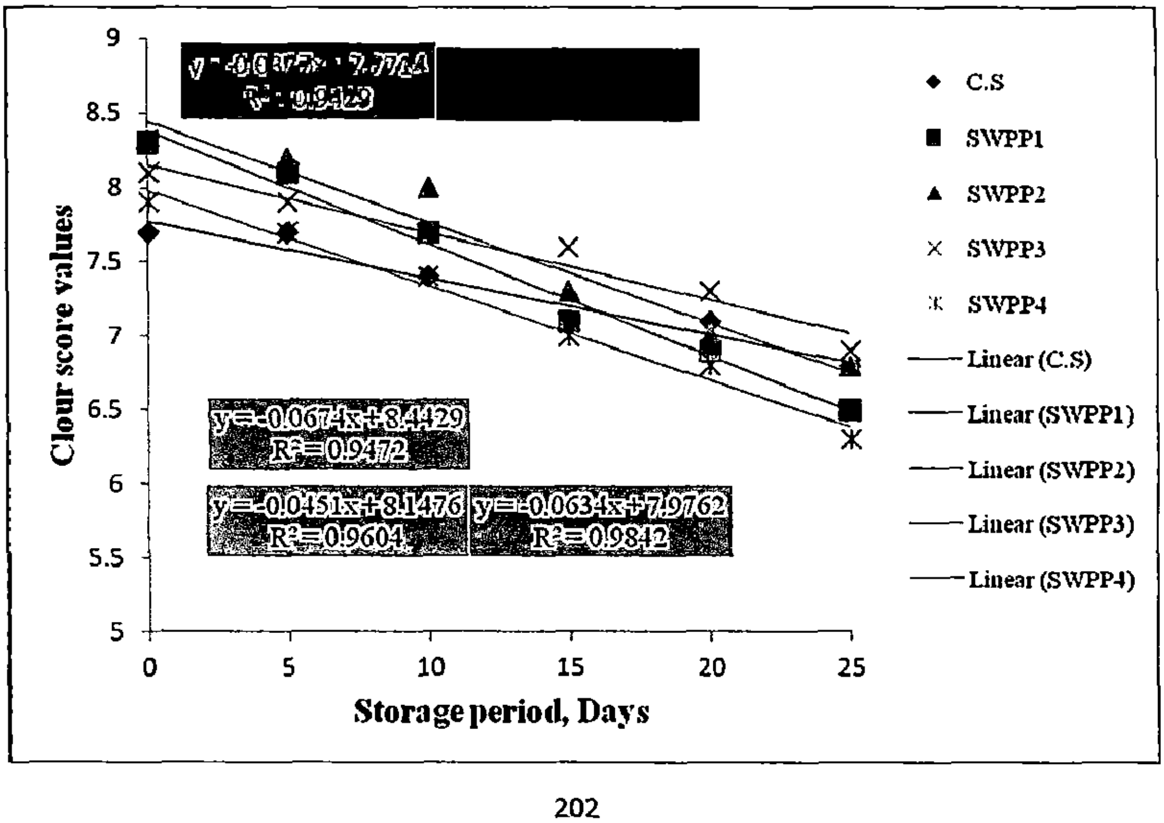



**Fig 4.69:** Regression analysis of colour of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.2 Aroma

The characteristic aroma of emulsion sausages mainly originates from the breakdown of carbohydrates, lipids and proteins through the action of microbial and endogenous meat enzymes (Kaban and Kaya, 2009). The development of aroma is also influenced by several variables such as product formulation (especially spices), processing condition and starter culture (Stahnke, 1994; Lucke, 1998; Toldra *et al.*, 2001).

In the present study all the emulsion sausages samples incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) had score values of aroma between '8' to '9'. Tables 4.86, 4.88 and 4.90 present the results of perceived aroma of emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively during refrigerated storage. Different levels of whey protein products did not significantly ($p<0.05$) affect the aroma of emulsion sausages (ANOVA Tables 4.87, 4.89 and 4.91), but the interaction between whey protein products and storage period significantly ($p<0.05$) affected the aroma score values. The enzymatic and chemical phenomena involved in flavour generation include carbohydrate fermentation, lipolysis, proteolysis, lipid oxidation and amino acid catabolism. The biochemical changes can be accelerated by the exogenous addition of different microorganism and/or enzymes to the initial formulation (Lucke, 2000; Talon *et al.*, 2002; Toldra *et al.*, 2001). During refrigerated storage the score values of aroma significantly ($p<0.05$) decreased end of 25$^{th}$ day of storage it was between of 6.3-7.0, 5.8-6.2 and 6.1-6.3 for emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively. The results of aroma of emulsion sausages during refrigerated storage (0°C) also have been demonstrated in Figure 4.70, 4.42 and 4.74. Kandeepan and Biswas (2007a) reported that the aroma scores of buffalo meat decreased significantly ($p<0.05$) with increase in storage period. Similar results were found by Kala *et al.*, (2007), who advocated that the aroma scores values of chicken emulsion patties was found to decrease progressively during refrigerated storage at 4°C.

Figures 4.71, 4.73 and 4.75 represent the linear regression of aroma score values of emulsion sausages incorporated with whey protein concentrate, isolate and powder respectively during refrigerated storage period. The equation of regression line and correlation coefficients were shown on the regression graph. The negative sign in the coefficients of x explain that there was constant decrease of aroma score values during storage period. The values of $R^2$ for all samples at levels 1, 2, 3 and 4 % of whey protein concentrate, isolate and powder were between 0.9589 – 0.9954, which shows relation between aroma of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

**Table 4.86:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Aroma score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 8.4± 0.13a | 8.0± 0.11 | 7.5± 0.11 | 6.8± 0.16 | 6.4± 0.28 | 6.1± 0.19b |
| **$Swpc_1$** | 8.4± 0.25a | 8.1± 0.11 | 7.5± 0.14 | 7.1± 0.22 | 6.7± 0.18 | 6.3± 0.33bc |
| **$Swpc_2$** | 8.4± 0.15a | 8.0± 0.18 | 7.7± 0.18 | 7.3± 0.11 | 7.0± 0.18 | 6.4± 0.66bc |
| **$Swpc_3$** | 8.5± 0.20a | 8.2± 0.08 | 7.8± 0.18 | 7.5± 0.16 | 7.2± 0.24 | 7.0± 0.35c |
| **$Swpc_4$** | 8.3± 0.26a | 7.9± 0.08 | 7.6± 0.14 | 7.2± 0.64 | 6.9± 0.19 | 6.4± 0.35bc |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.87:** ANOVA of aroma of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 81.45 | 0.546644 | | |
| **Replicate** | 4 | 0.739477 | 0.184869 | 2.424287 | 2.46 |
| **FA** | 4 | 3.636811 | 0.909203 | 11.92284 | 2.46 |
| **FB** | 5 | 67.42721 | 13.48544 | 176.8416 | 2.3 |
| **Comb(A*B)** | 29 | 2.226453 | 0.076774 | 1.00678 | 1.63 |
| **Error/Res** | 107 | 8.15952 | 0.076257 | | |
| **LSD** | 0.345808 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.70:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein concentrate

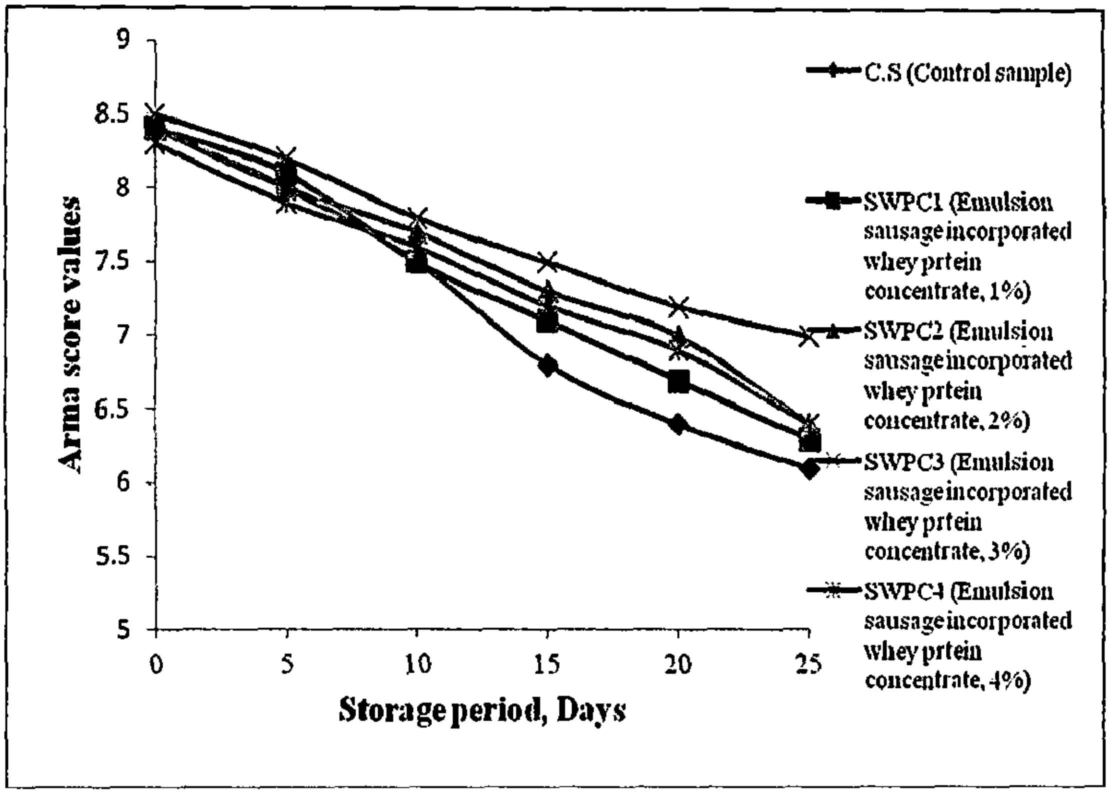



**Fig 4.71:** Regression analysis of aroma of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

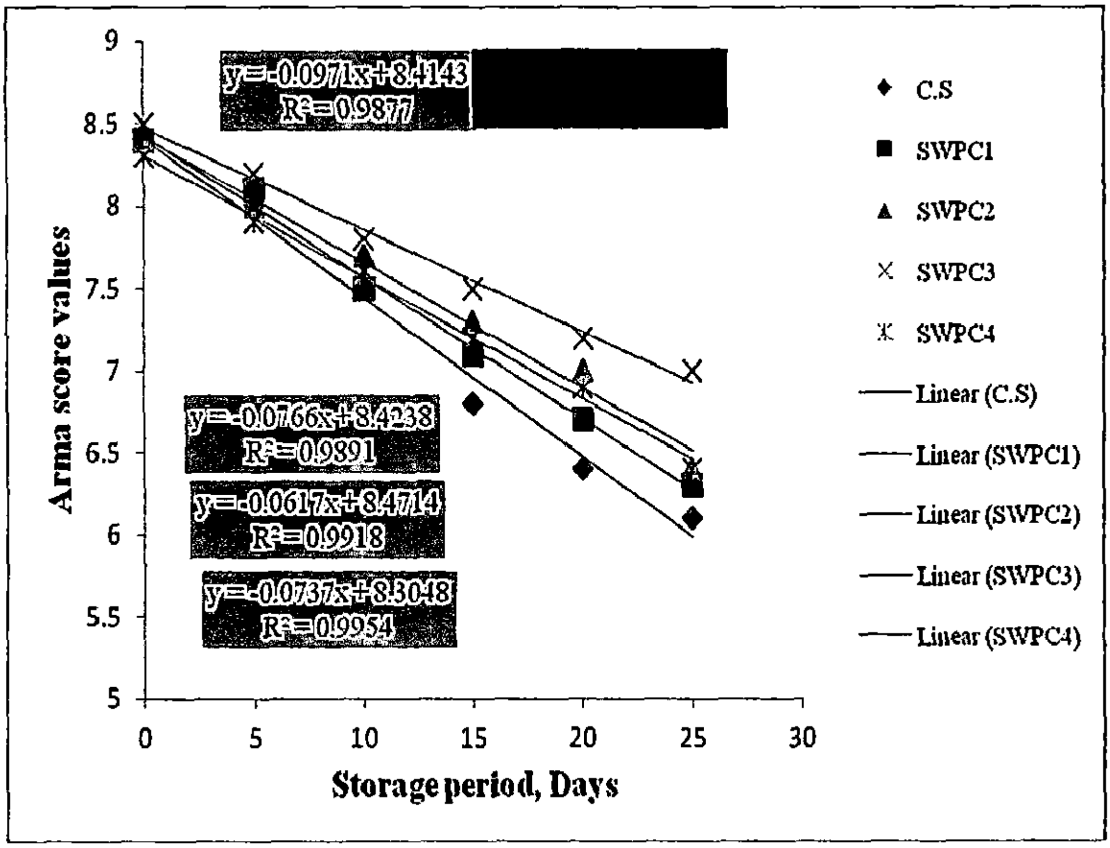


**Table 4.88:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Aroma score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 8.4± 0.13a | 8.0± 0.11 | 7.5± 0.11 | 6.8± 0.16 | 6.4± 0.28 | 6.1± 0.19b |
| **Swpi$_1$** | 8.0± 0.89ab | 7.7± 0.16 | 7.3± 0.08 | 6.6± 0.28 | 6.2± 0.08 | 5.9± 0.11c |
| **Swpi$_2$** | 8.0± 0.08ab | 7.7± 0.15 | 7.2± 0.11 | 6.5± 0.33 | 6.2± 0.20 | 5.8± 0.13c |
| **Swpi$_3$** | 8.1± 0.08ab | 7.8± 0.12 | 7.5± 0.10 | 7.0± 0.35 | 6.5± 0.20 | 6.1± 0.19b |
| **Swpi$_4$** | 8.1± 0.08ab | 7.7± 0.11 | 7.4± 0.17 | 7.0± 0.11 | 6.6± 0.15 | 6.2± 0.30b |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpi$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.89:** ANOVA of aroma of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 91.6923 | 0.615385 | | |
| **Replicate** | 4 | 0.443844 | 0.110961 | 2.963376 | 2.46 |
| **FA** | 4 | 2.598384 | 0.649596 | 17.34842 | 2.46 |
| **FB** | 5 | 84.0742 | 16.81484 | 449.065 | 2.3 |
| **Comb(A*B)** | 29 | 1.0132 | 0.034938 | 0.933069 | 1.63 |
| **Error/Res** | 107 | 4.00652 | 0.037444 | | |
| **LSD** | 0.242319 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.72:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein isolate

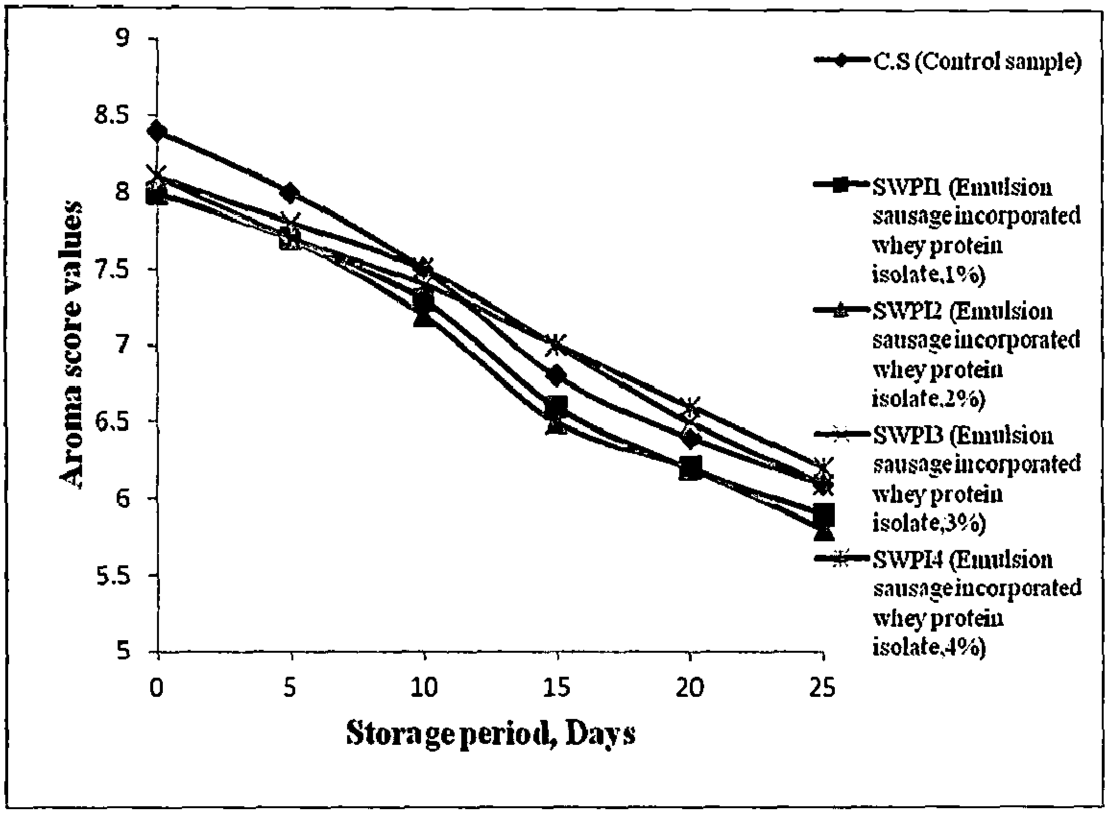



**Fig 4.73:** Regression analysis of aroma of emulsion sausages incorporated with different levels whey protein isolate during refrigerated storage (0°C)

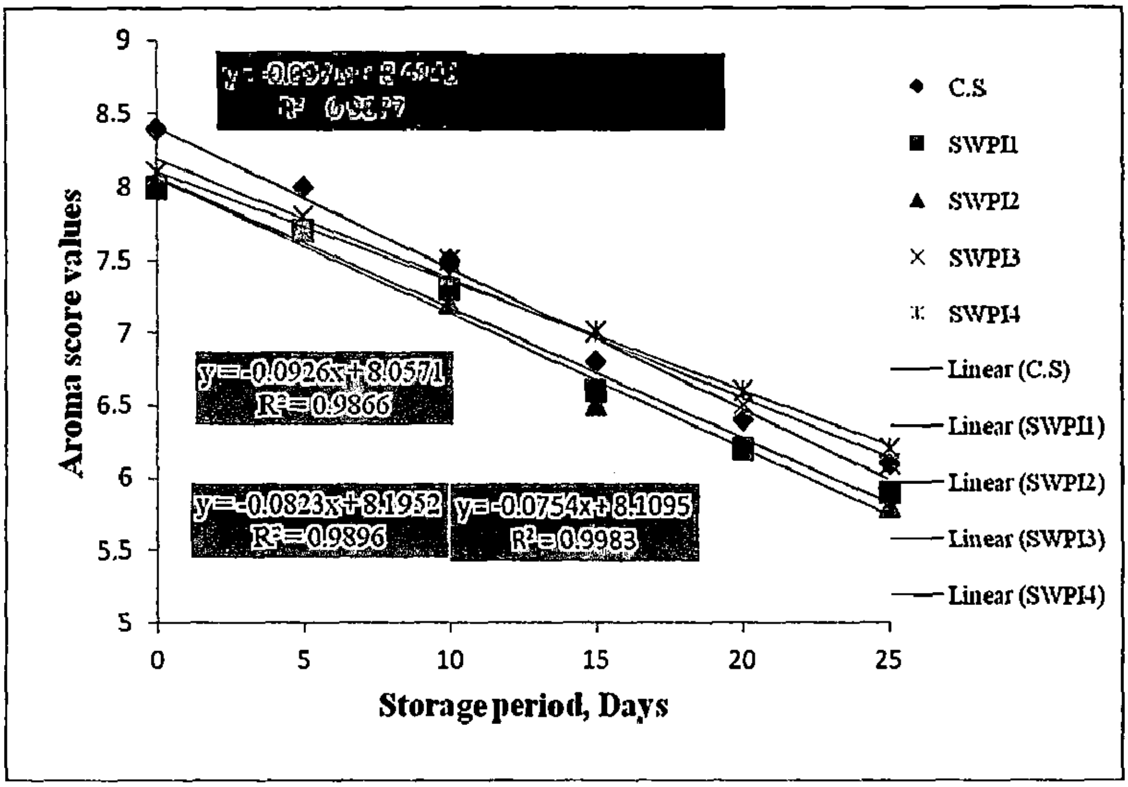


**Table 4.90:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Aroma score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 8.4± 0.13a | 8.0± 0.11 | 7.5± 0.11 | 6.8± 0.16 | 6.4± 0.28 | 6.1± 0.19b |
| **Swpp$_1$** | 8.4± 0.12a | 7.9± 0.08 | 7.6± 0.14 | 7.1± 0.13 | 6.8± 0.25 | 6.2± 0.19b |
| **Swpp$_2$** | 8.4± 0.16a | 8.1± 0.19 | 7.6± 0.14 | 7.0± 0.18 | 6.6± 0.35 | 6.3± 0.11bc |
| **Swpp$_3$** | 8.3± 0.01a | 8.2± 0.08 | 7.7± 0.16 | 7.2± 0.18 | 6.7± 0.14 | 6.3± 0.20bc |
| **Swpp$_4$** | 8.2± 0.01a | 8.1± 0.19 | 7.8± 0.18 | 7.1± 0.25 | 6.7± 0.25 | 6.1± 0.16b |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpp$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.91:** ANOVA of aroma of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 93.52083 | 0.627657 | | |
| **Replicate** | 4 | 0.315311 | 0.078828 | 2.250224 | 2.46 |
| **FA** | 4 | 0.452057 | 0.113014 | 3.226121 | 2.46 |
| **FB** | 5 | 88.11976 | 17.62395 | 503.0955 | 2.3 |
| **Comb(A*B)** | 29 | 1.200687 | 0.041403 | 1.181895 | 1.63 |
| **Error/Res** | 107 | 3.74832 | 0.035031 | | |
| **LSD** | 0.234381 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.74:** Effect of refrigerated storage (0°C) on aroma of emulsion sausages incorporated with different levels of whey protein powder

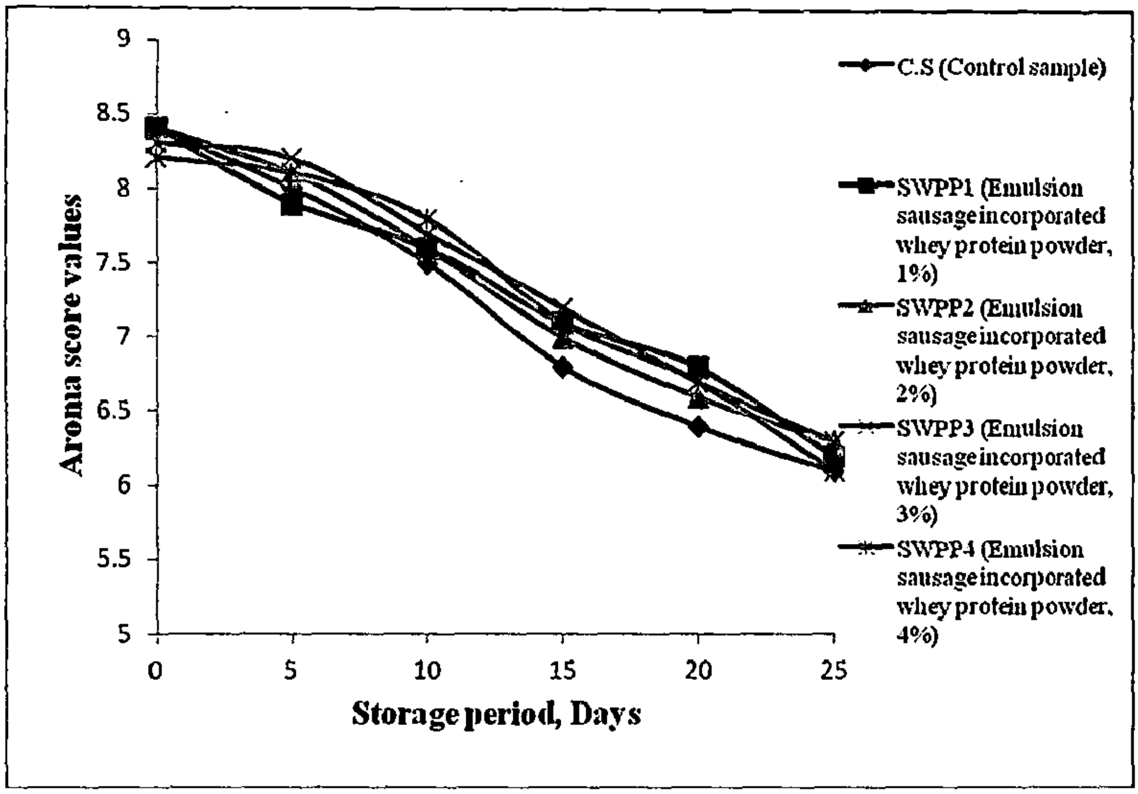
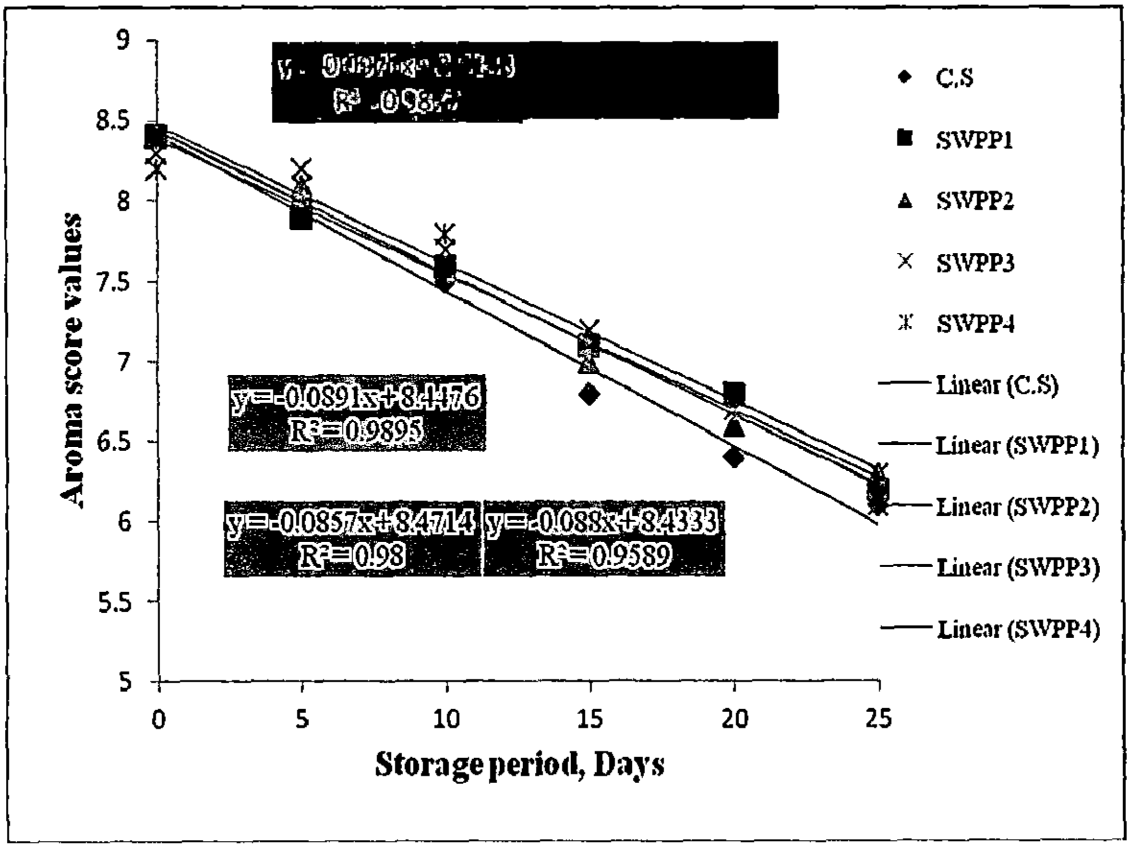



**Fig 4.75:** Regression analysis of aroma of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.3 Texture

Texture is a predominant of the element of the quality and acceptability of foods. It is perceived from sensory impressions of the physical properties of a material, its nature, composition and behaviour on deformation received from senses of touch, sight and hearing (Mathoniere *et al.*, 2000). Tables 4.92, 4.94 and 4.96 present the results of evaluation of texture of emulsion sausages during refrigerated storage incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) respectively. The samples incorporated with whey protein concentrate at the level of 4% and whey protein isolate at level of 1% and whey powder at the level of 4% received highest score values of 8.7, 8.6 and 8.4 respectively. The samples incorporated with whey protein concentrate at the level of 1%, isolate at the level of 4% and whey powder at the level of 1% had lowest score values as 8.1, 8.2 and 8.3 respectively. Different levels of whey protein products significantly ($p<0.05$) increased the texture score values of emulsion sausages at the levels (1, 2, 3 and 4%) whey protein concentrate, isolate and powder (ANOVA Tables 4.93, 4.95 and 4.97). When the pH is lowered, protein starts to coagulate and it brings change in texture and colour (Ngapo *et al.*, 1996; Barbut 2005). Lower pH in cooked salami type sausages resulted in more disruption of protein coagulates formed during heating (Barbut, 2006).

During the refrigerated storage (0°C), the score values of texture of emulsion sausages incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) was found to significantly ($p<0.05$) decrease. The mean score values of texture on 25$^{th}$ day of storage for all samples were between of 5.5-6.6. Figure 4.76, 4.78 and 4.80 represent the profile of texture score values of emulsion sausages (controlled and treated) during refrigerated storage. Kandeepan *et al.*, (2010), they reported that ambient storage significantly ($p<0.05$) decreased the score values of texture of meat keema. It should be noted that the changes of other chemical contents such as protein, moisture, fat collagen and pH value during storage might be due to the change of texture of cooked sausages (Dong *et al.*, 2007). The $R^2$ for all samples at different levels of whey protein products were between 0.9239-0.9976, which shows relation between texture of the samples and storage period were almost perfect and the graph may be approximated to a straight line (Figures 4.77, 4. 79 and 4.81).

e 4.92: Effect of refrigerated storage (0°C) on texture of emulsion sausages porated with different levels of whey protein concentrate

| nple ode | Texture score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| 's | 7.5± 0.07a | 7.2± 0.15 | 6.9± 0.23 | 6.4± 0.14 | 5.9± 0.13 | 5.5± 0.37c |
| 'pc1 | 8.1± 0.01b | 7.4± 0.26 | 7.2± 0.21 | 6.8± 0.21 | 6.4± 0.37 | 5.9± 0.35cd |
| 'pc2 | 8.5± 0.07bc | 8.2± 0.14 | 7.7± 0.19 | 7.2± 0.17 | 6.9± 0.59 | 6.2± 0.22d |
| 'pc3 | 8.4± 0.07bc | 8.3± 0.19 | 8.0± 0.19 | 7.4± 0.35 | 6.6± 0.20 | 5.7± 0.17cd |
| 'pc4 | 8.7± 0.10bc | 8.5± 0.32 | 8.1± 0.25 | 7.8± 0.18 | 7.2± 0.24 | 6.6± 0.15e |

s are mean of five replicates ±SD; Means with different letters in a column differ significantly 05), Cs= Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of protein concentrate (1, 2, 3 and 4%)

le 4.93: ANOVA of texture of emulsion sausages incorporated with different s of whey protein concentrate during refrigerated storage

| ource | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 36.2126 | 0.243038 | | |
| plicate | 4 | 0.380933 | 0.095233 | 3.000579 | 2.46 |
| FA | 4 | 11.11827 | 2.779567 | 87.57763 | 2.46 |
| FB | 5 | 21.1958 | 4.23916 | 133.566 | 2.3 |
| nb(A*B) | 29 | 0.502533 | 0.017329 | 0.545988 | 1.63 |
| ror/Res | 107 | 3.396 | 0.031738 | | |
| LSD | 0.223094 | | | | |

Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, = Least significance difference, FA = Different levels of whey protein incorporated, Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.76:** Effect of refrigerated storage (0°C) on texture of emulsion sausages incorporated with different levels of whey protein concentrate

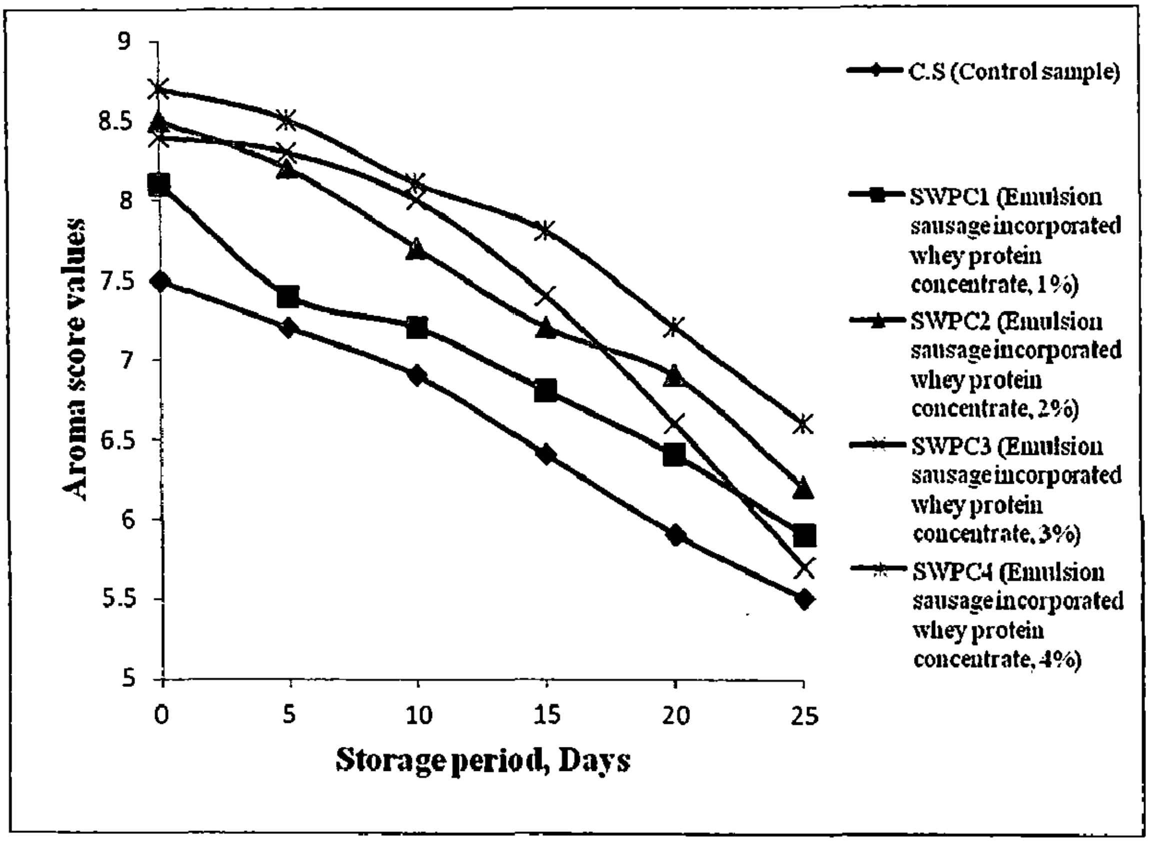



**Fig 4.77:** Regression analysis of texture of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

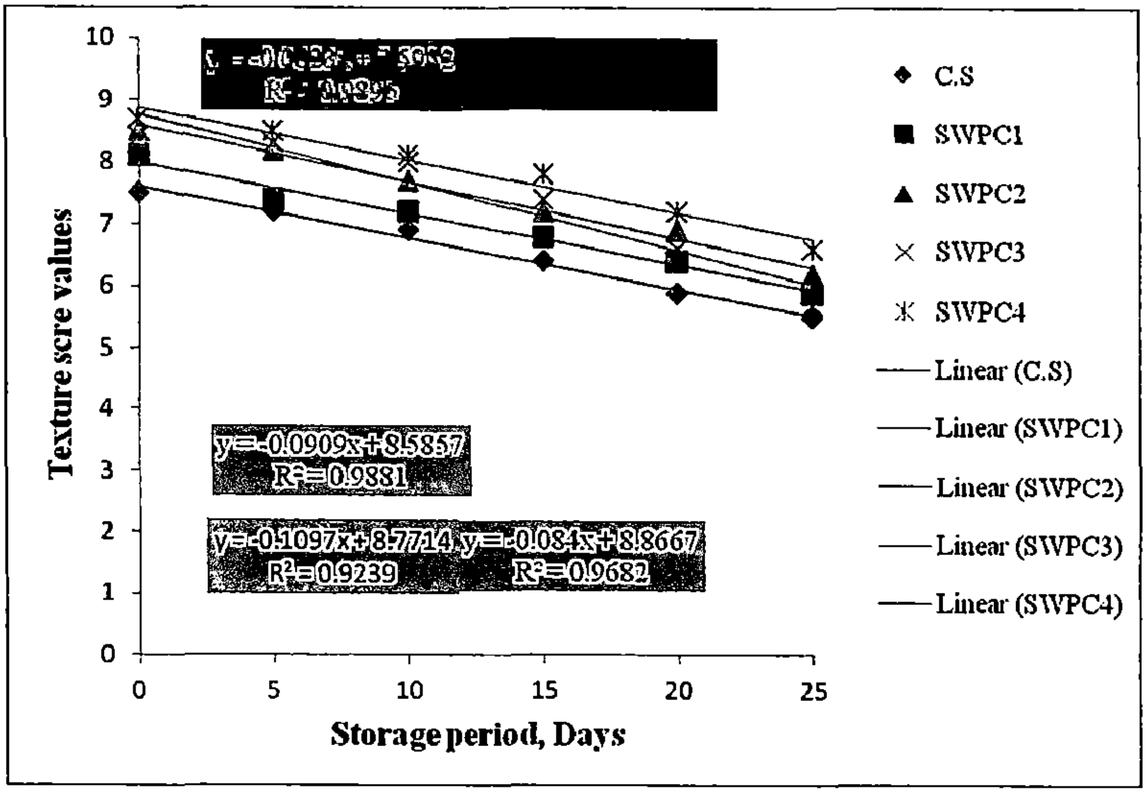


**Table 4.94:** Effect of refrigerated storage (0°C) on texture of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Texture score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.5± 0.07a | 7.2± 0.15 | 6.9± 0.23 | 6.4± 0.14 | 5.9± 0.13 | 5.5± 0.37c |
| $Swpi_1$ | 8.6± 0.05b | 8.1± 0.19 | 7.4± 0.21 | 6.9± 0.17 | 6.5± 0.35 | 5.9± 0.35cd |
| $Swpi_2$ | 8.3± 0.89bc | 7.8± 0.11 | 7.5± 0.14 | 7.1± 0.20 | 6.7± 0.17 | 6.1± 0.15d |
| $Swpi_3$ | 8.4± 0.83bc | 7.9± 0.13 | 7.3± 0.28 | 6.9± 0.17 | 6.4± 0.36 | 5.8± 0.18cd |
| $Swpi_4$ | 8.2± 0.05bc | 7.8± 0.10 | 7.4± 0.31 | 6.7± 0.14 | 6.1± 0.16 | 5.5± 0.32c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.95:** ANOVA of texture of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 120.8483 | 0.811063 | | |
| Replicate | 4 | 0.61 | 0.1525 | 3.023999 | 2.46 |
| FA | 4 | 9.349333 | 2.337333 | 46.34816 | 2.46 |
| FB | 5 | 103.7139 | 20.74279 | 411.3192 | 2.3 |
| Comb(A*B) | 29 | 2.389067 | 0.082382 | 1.633586 | 1.63 |
| Error/Res | 107 | 5.396 | 0.05043 | | |
| LSD | 0.281216 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.78:** Effect of refrigerated storage (0°C) on texture of emulsion sausages incorporated with different levels of whey protein isolate

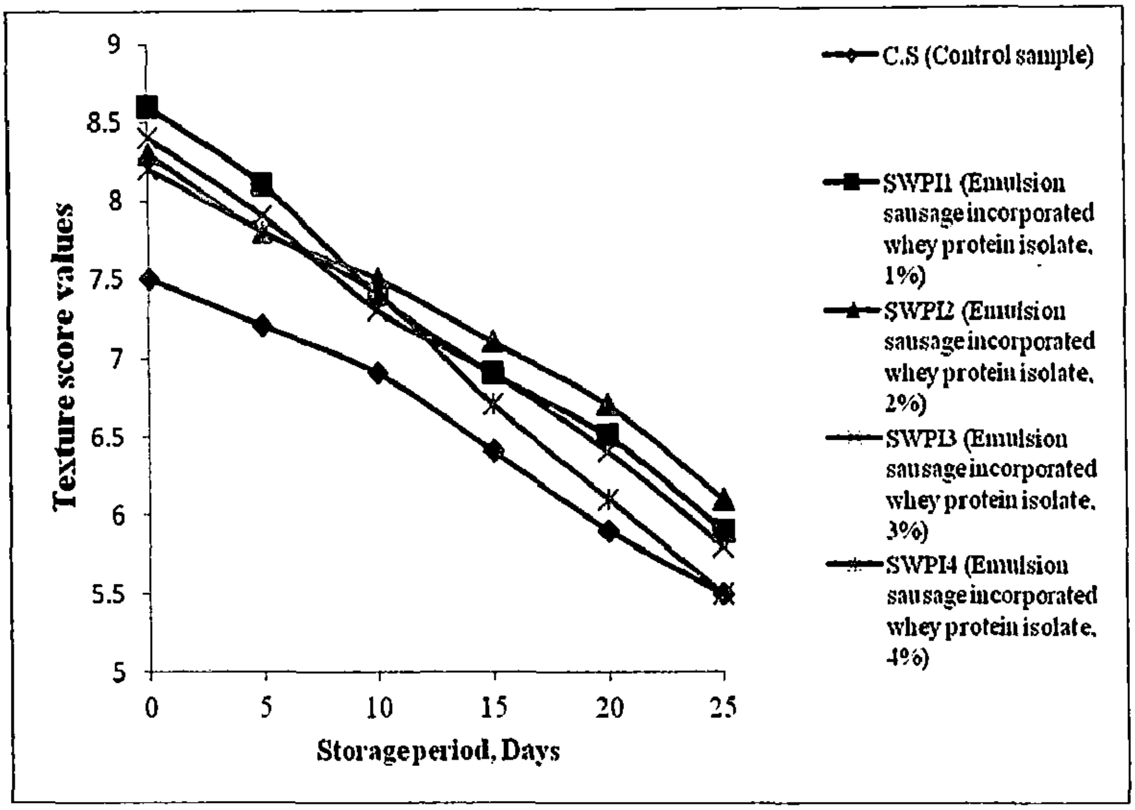



**Fig 4.79:** Regression analysis of texture of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

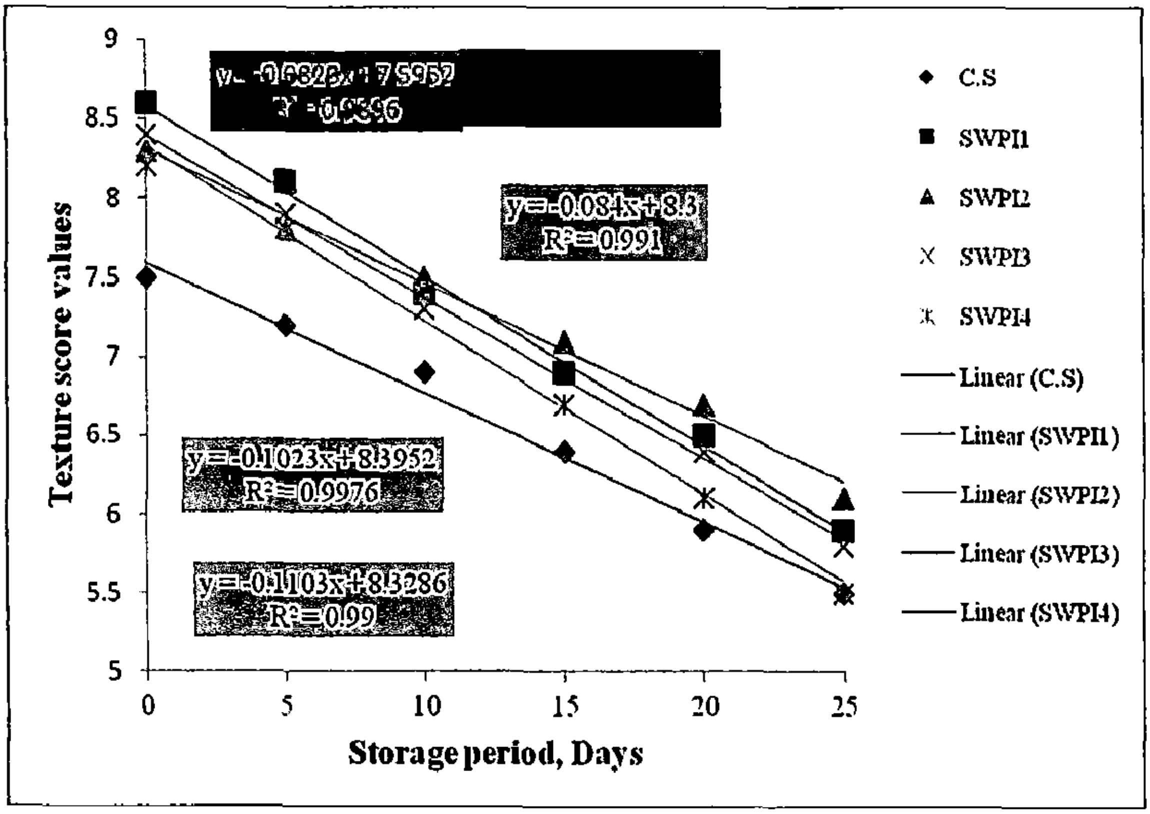


**Table 4.96:** Effect of refrigerated storage (0°C) on texture of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Texture score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.5± 0.07a | 7.2± 0.15 | 6.9± 0.23 | 6.4± 0.14 | 5.9± 0.13 | 5.5± 0.37c |
| $Swpp_1$ | 8.3± 0.02b | 8.0± 0.35 | 7.7± 0.21 | 7.5± 0.36 | 7.0± 0.14 | 6.6± 0.39d |
| $Swpp_2$ | 8.4± 0.02b | 8.2± 0.15 | 7.8± 0.14 | 7.2± 0.26 | 6.7± 0.16 | 6.0± 0.18e |
| $Swpp_3$ | 8.4± 0.01b | 8.1± 0.20 | 7.6± 0.80 | 7.1± 0.16 | 6.6± 0.20 | 6.2± 0.18e |
| $Swpp_4$ | 8.4± 0.20b | 8.0± 0.55 | 7.7± 0.18 | 7.0± 0.38 | 6.5± 0.32 | 6.1± 0.21e |

Values are mean of five replicates ±SD;
Means with different letters in a column differ significantly ($p<0.05$)
Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.97:** ANOVA of texture of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 105.1394 | 0.705634 | | |
| Replicate | 4 | 0.095633 | 0.023908 | 0.363758 | 2.46 |
| FA | 4 | 16.31422 | 4.078555 | 62.05392 | 2.46 |
| FB | 5 | 79.907 | 15.9814 | 243.1519 | 2.3 |
| Comb(A*B) | 29 | 1.8855 | 0.065017 | 0.989217 | 1.63 |
| Error/Res | 107 | 7.03268 | 0.065726 | | |
| LSD | 0.321043 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.80:** Effect of refrigerated storage (0°C) on texture of emulsion sausages incorporated with different levels of whey protein powder

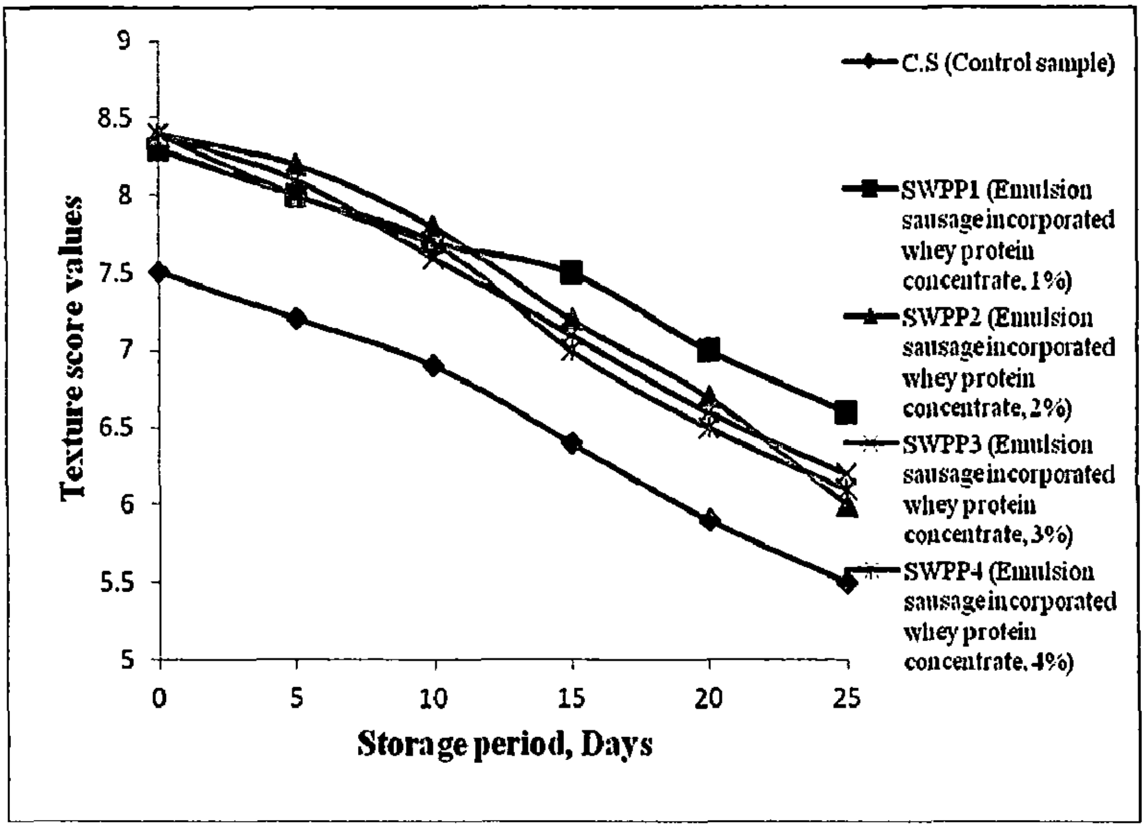
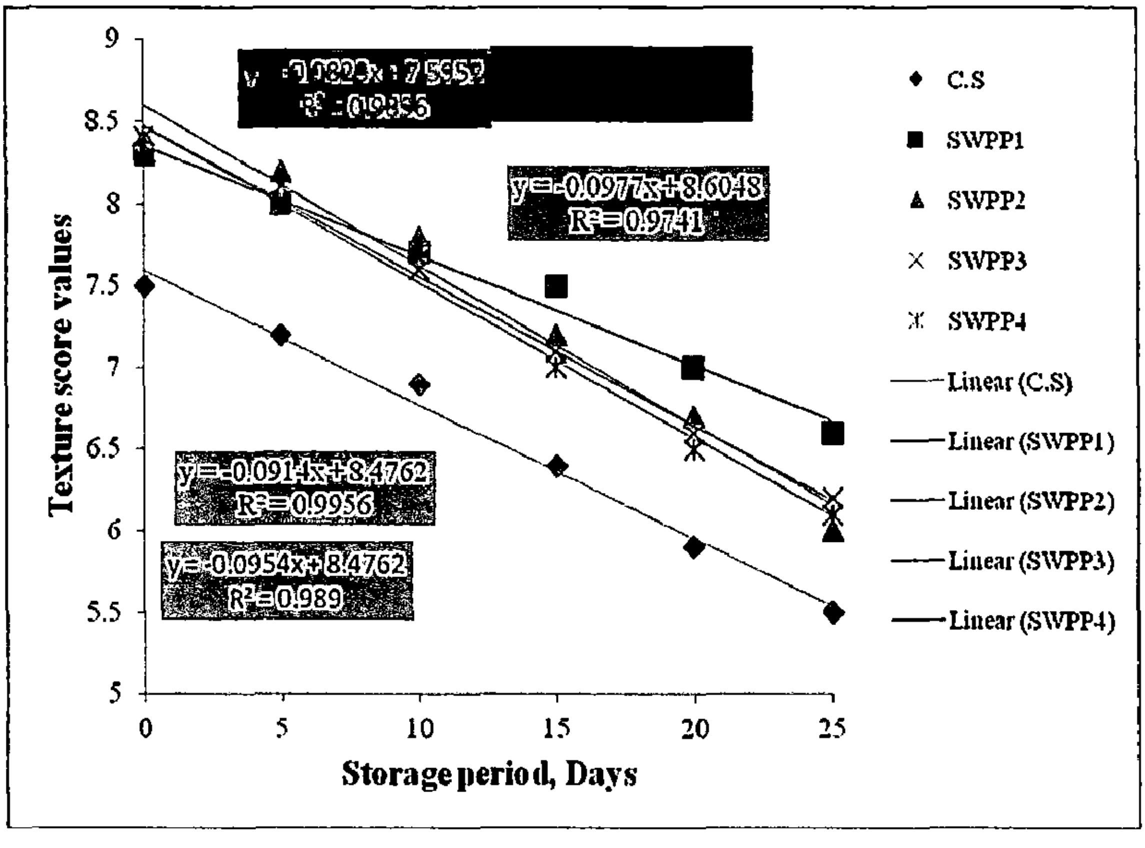



**Fig 4.81:** Regression analysis of texture of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.4 Taste

Tables 4.98, 4.100 and 4.102 presents the results of sensory taste of emulsion sausage samples incorporated with whey protein concentrate isolate and powder with different levels (1, 2, 3 and 4%) during refrigerated storage. All the emulsion sausages samples in fresh condition had the score values of taste between 8.1 to 8.7. It represented condition between liked very much and liked extremely. The score value of control sample was 7.9. Different levels of whey protein concentrate, isolate and whey protein powder significantly ($p<0.05$) increased the taste score values in fresh condition of emulsion sausages samples as compared to the control sample. Also the interaction between whey protein products and storage period significantly ($p<0.05$) affected the taste score values of emulsion sausage samples (Tables 4.99, 4.101 and 4.103). At the end of storage period (on $25^{th}$ day), there was significant ($p<0.05$) decrease in score values of taste of different whey products samples (Figure 4.82, 4.84 and 4.86). This might be due to different pH and TBA number values. This finding is in agreement with Balev *et al.*, (2011), who advocated that the sensory evaluated taste decreased steadily during chilled storage. The means score values of taste on $25^{th}$ day of storage were between of 6.9-7.5 for all samples. Sample treated with whey protein concentrate at the level of 3% had highest scored value (7.5) for the taste among all samples. While lowest score values were given to the samples incorporated whey with the level of 3% whey powder.

Figure (4.83, 4.85 and 4.87) represent the linear regression of taste score values of emulsion sausages incorporated with whey protein concentrate, isolate and powder with different levels of 1, 2, 3 and 4% during refrigerated storage period. The equation of regression line and correlation coefficients were shown on the regression graph. The negative sign in the coefficients of x explain that there was constant decrease of taste score values during storage period. The values of $R^2$ for all sausages samples prepared with whey protein concentrate with different levels (1, 2, 3 and 4%) were found in range of 0.950-0.996, for whey protein isolate the range was of 0.950–0.989 and that for whey protein powder the range was 0.9623-0.9965. The values of $R^2$ show relation between taste of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

**Table 4.98:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Taste score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.9± 0.08a | 7.7± 0.19 | 7.5± 0.21 | 7.3± 0.18 | 7.0± 0.11 | 6.9± 0.08c |
| $Swpc_1$ | 8.1± 0.08b | 8.0± 0.11 | 7.7± 0.17 | 7.5± 0.28 | 7.2± 0.13 | 7.1± 0.13d |
| $Swpc_2$ | 8.6± 0.07bc | 8.4± 0.11 | 8.1± 0.16 | 7.8± 0.14 | 7.6± 0.17 | 7.3± 0.22de |
| $Swpc_3$ | 8.7± 0.05bc | 8.5± 0.21 | 8.3± 0.15 | 8.0± 0.17 | 7.8± 0.21 | 7.5± 0.19de |
| $Swpc_4$ | 8.3± 0.13b | 8.3± 0.18 | 8.1± 0.12 | 7.9± 0.21 | 7.7± 0.15 | 7.4± 0.22de |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.99:** ANOVA of taste of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 36.2126 | 0.243038 | | |
| Replicate | 4 | 0.380933 | 0.095233 | 3.000579 | 2.46 |
| FA | 4 | 11.11827 | 2.779567 | 87.57763 | 2.46 |
| FB | 5 | 21.1958 | 4.23916 | 133.566 | 2.3 |
| Comb(A*B) | 29 | 0.502533 | 0.017329 | 0.545988 | 1.63 |
| Error/Res | 107 | 3.396 | 0.031738 | | |
| LSD | 0.223094 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.82:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein concentrate

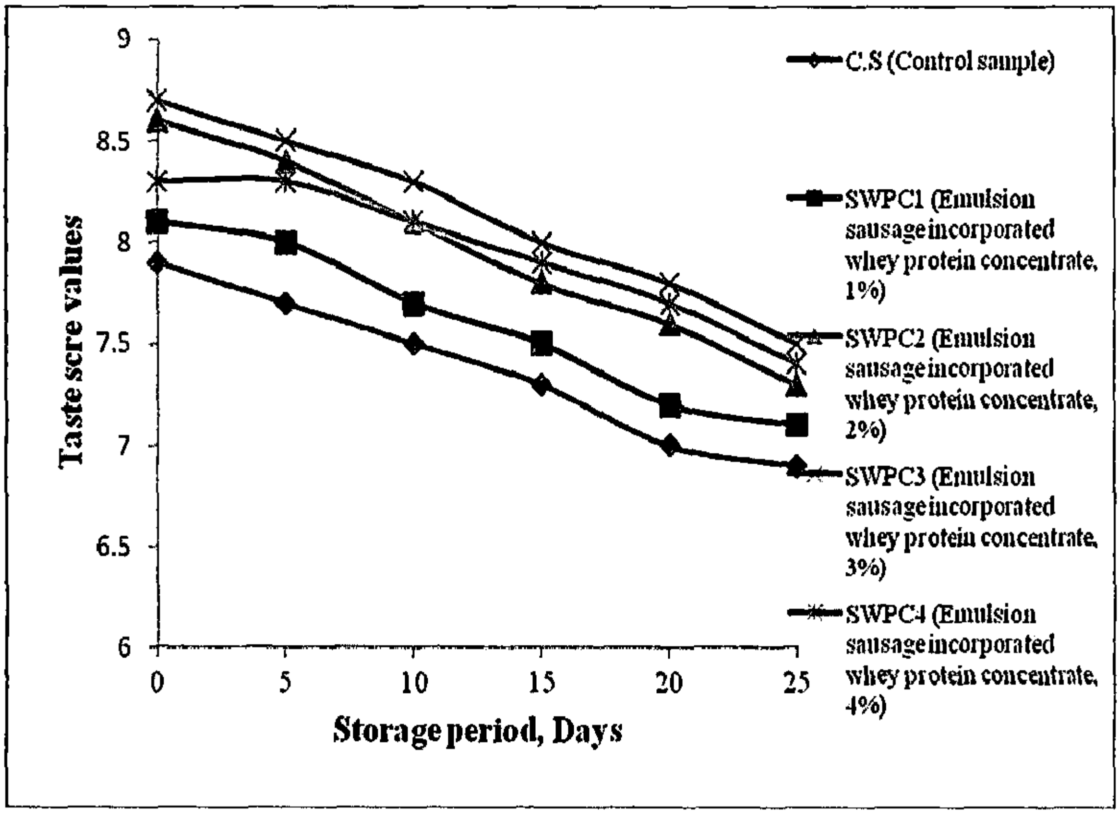



**Fig 4.83:** Regression analysis of taste of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

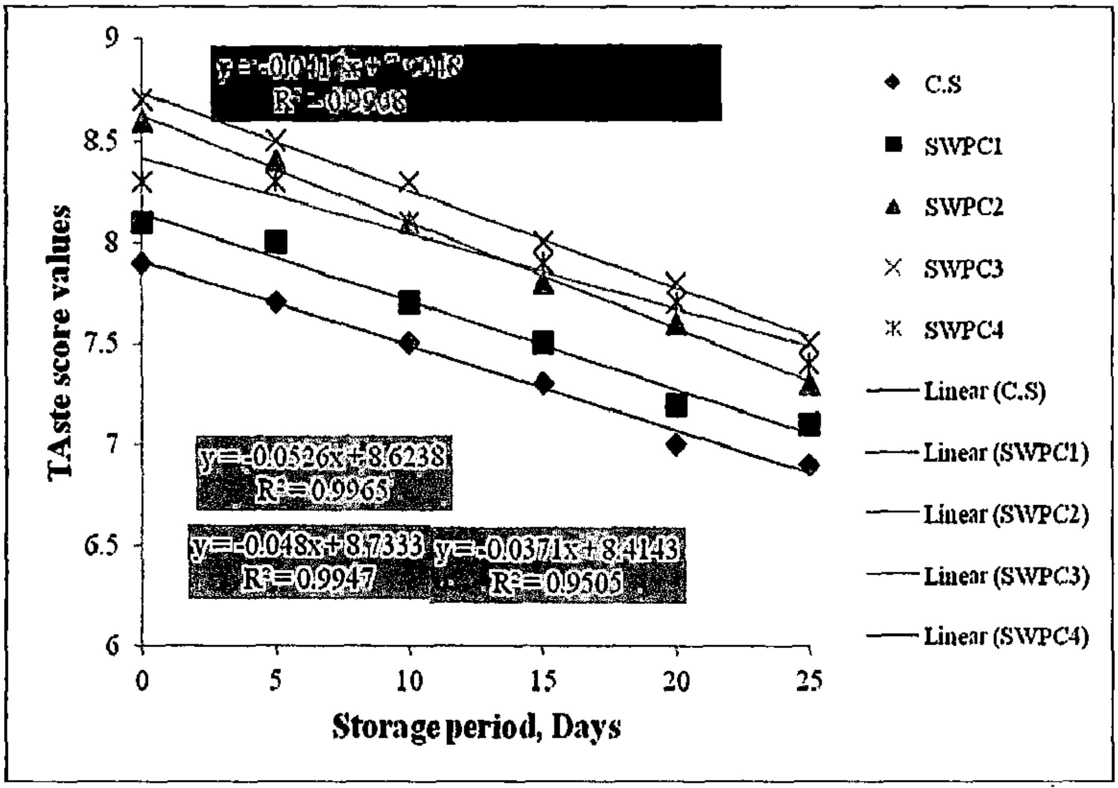


**Table 4.100:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Taste score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.9± 0.08a | 7.7± 0.19 | 7.5± 0.21 | 7.3± 0.18 | 7.0± 0.11 | 6.9± 0.08c |
| $Swpi_1$ | 8.3± 0.08b | 8.1± 0.14 | 7.9± 0.13 | 7.7± 0.10 | 7.4± 0.13 | 7.1± 0.19d |
| $Swpi_2$ | 8.2± 0.05b | 7.9± 0.13 | 7.7± 0.11 | 7.7± 0.10 | 7.5± 0.23 | 7.3± 0.22e |
| $Swpi_3$ | 8.2± 0.04b | 8.0± 0.35 | 7.9± 0.13 | 7.6± 0.10 | 7.5± 0.29 | 7.4± 0.32e |
| $Swpi_4$ | 8.1± 0.07b | 8.0± 0.28 | 7.8± 0.13 | 7.6± 0.20 | 7.3± 0.25 | 7.1± 0.17d |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.101:** ANOVA of taste of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 24.46673 | 0.164206 | | |
| Replicate | 4 | 0.1004 | 0.0251 | 0.666758 | 2.46 |
| FA | 4 | 3.0564 | 0.7641 | 20.29759 | 2.46 |
| FB | 5 | 16.62753 | 3.325507 | 88.33893 | 2.3 |
| Comb(A*B) | 29 | 0.7548 | 0.026028 | 0.691398 | 1.63 |
| Error/Res | 107 | 4.028 | 0.037645 | | |
| LSD | 0.242967 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.84:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein isolate

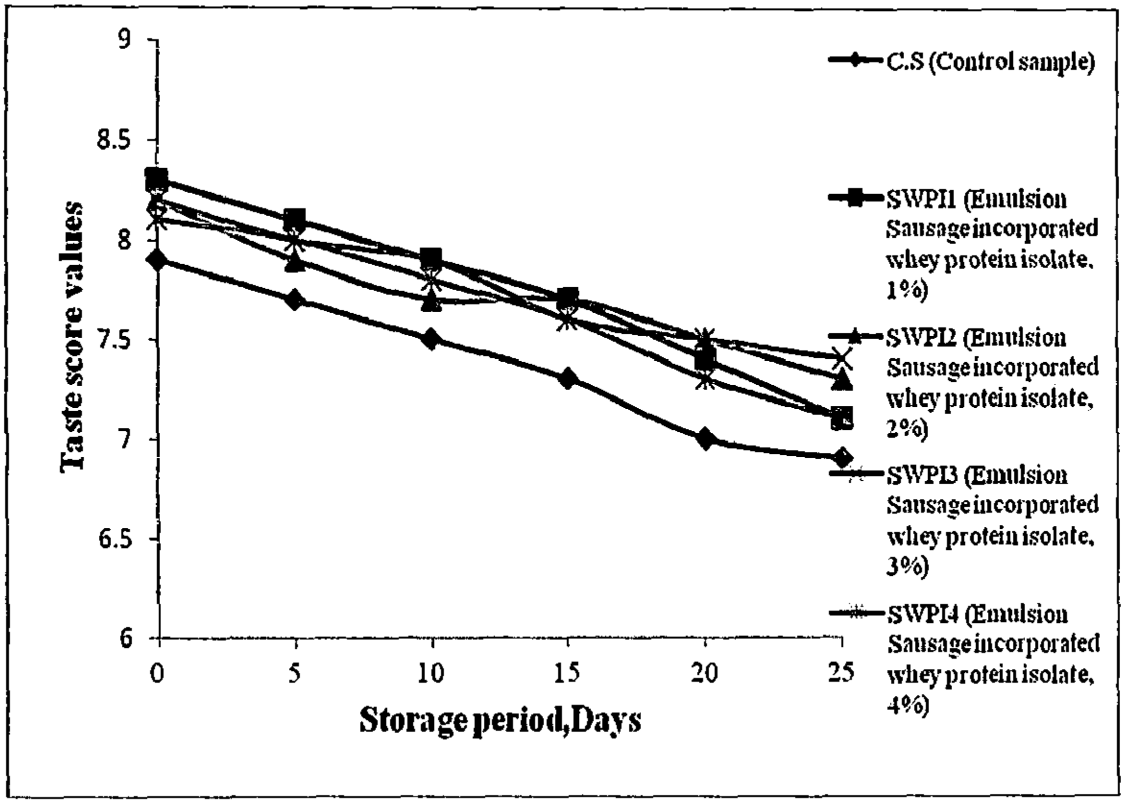



**Fig 4.85:** Regression analysis of taste of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

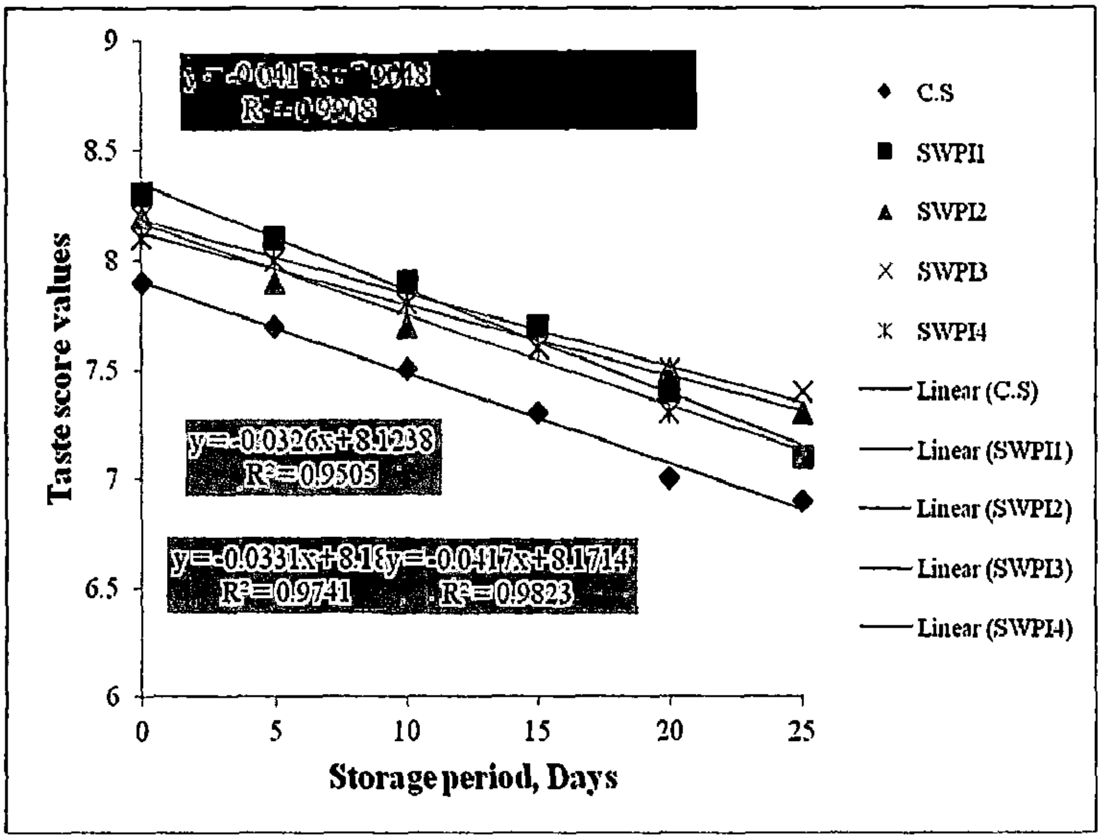


**Table 4.102:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Taste score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.9± 0.08a | 7.7± 0.19 | 7.5± 0.21 | 7.3± 0.18 | 7.0± 0.11 | 6.9± 0.08c |
| $Swpp_1$ | 8.5± 0.07b | 8.2± 0.21 | 8.0± 0.211 | 7.8± 0.14 | 7.4± 0.25 | 7.1± 0.20d |
| $Swpp_2$ | 8.0± 0.08b | 7.9± 0.11 | 7.8± 0.29 | 7.5± 0.16 | 7.3± 0.19 | 7.0± 0.15d |
| $Swpp_3$ | 8.3± 0.08b | 8.2± 0.14 | 7.9± 0.15 | 7.4± 0.29 | 7.2± 0.23 | 6.9± 0.08c |
| $Swpp_4$ | 8.4± 0.08b | 8.2± 0.15 | 7.9± 0.38 | 7.6± 0.22 | 7.2± 0.20 | 7.1± 0.19d |

*Values are mean of five replicates ±SD; Means with different letters in a column differ significantly* ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.103:** ANOVA of taste of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 36.88773 | 0.247569 | | |
| **Replicate** | 4 | 0.365067 | 0.091267 | 2.340732 | 2.46 |
| **FA** | 4 | 3.3984 | 0.8496 | 21.78984 | 2.46 |
| **FB** | 5 | 28.23493 | 5.646987 | 144.8292 | 2.3 |
| **Comb(A*B)** | 29 | 1.0824 | 0.037324 | 0.957259 | 1.63 |
| **Error/Res** | 107 | 4.172 | 0.038991 | | |
| **LSD** | 0.247272 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.86:** Effect of refrigerated storage (0°C) on taste of emulsion sausages incorporated with different levels of whey protein powder

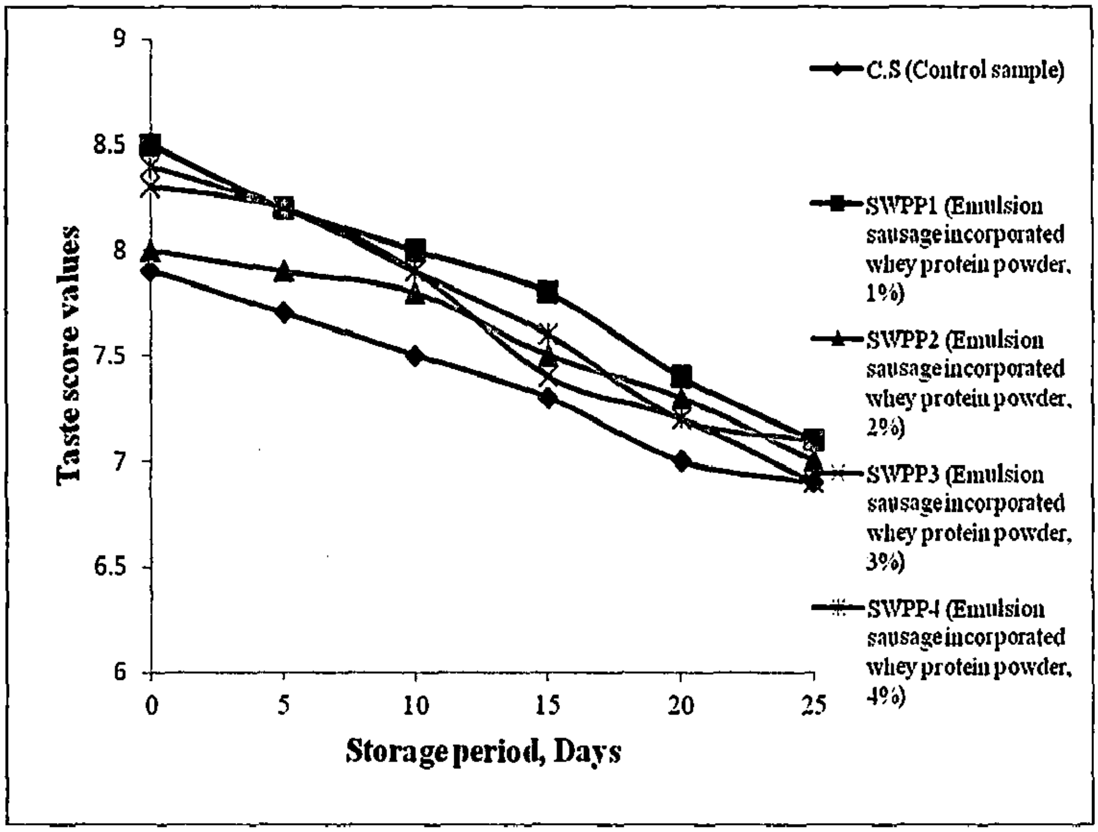
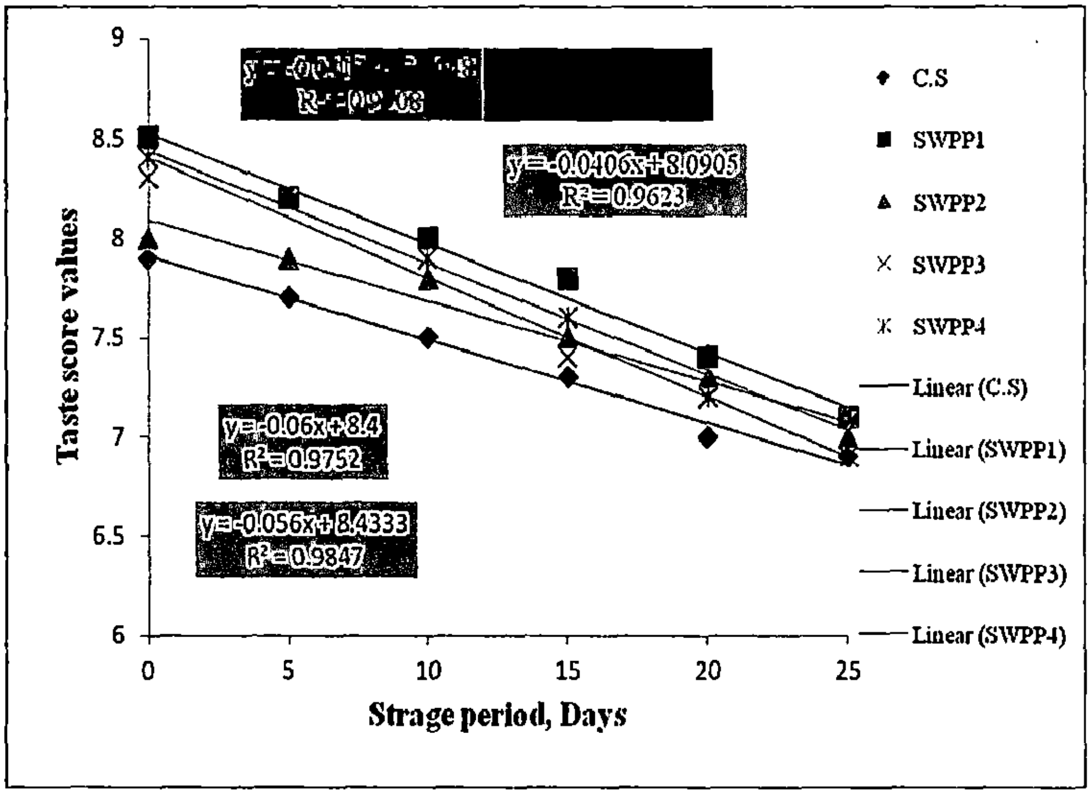



**Fig 4.87:** Regression analysis of taste of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.5 Mouth coating

Tables 4.104, 4.106 and 4.108 present the results of evaluation of mouth coating of emulsion sausages incorporated with whey protein concentrate isolate and powder with different levels (1, 2, 3 and 4%) during refrigerated storage. All the emulsion sausages in fresh condition had the score values of mouth coating between 7.9 to 8.5. It represented condition between liked very much to like extremely. The score value of control sample was 8.1. Different levels of whey protein concentrate, isolate and whey protein powder significantly ($p<0.05$) affected score values of the mouth coating in fresh condition of emulsion sausage samples. The interaction between whey protein products and storage period did not significantly ($p<0.05$) affect the mouth coating score values (Tables 4.105, 4.107 and 4.109). During refrigerated storage at $0^{\circ}C$ the score values of mouth coating significantly ($p<0.05$) decreased. The means of all samples were found to be on 25$^{th}$ day of storage in between of 6.5-7.1. Sample incorporated with whey protein concentrate at the level of 3 and 4% significantly ($p<0.05$) scored the highest value for the mouth coating among all samples. While lowest score values were given to the sausage samples incorporated with 2% whey protein powder. Figure 4.88, 4.90 and 4.92 represented decreasing profile of mouth coating score values of emulsion sausages (controlled and treated) during refrigerated storage (0°C).

Figures (4.89, 4.91 and 4.93) represent the linear regression score values of mouth coating score of emulsion sausages incorporated with whey protein concentrate, isolate and powder with levels of 1, 2, 3 and 4% during refrigerated storage. The equation of regression line and correlation coefficients were represented on the regression graph. The negative sign in the coefficients of x explain that there was constant decrease of mouth coating score values during storage period. The values of $R^2$ for all emulsion sausage samples prepared with whey protein concentrate at the levels (1, 2, 3 and 4%) were in the range of 0.9512-0.9916, for whey protein isolate the range was 0.9434–0.9925 and for whey protein powder the range was 0.949-0.9965. It was predicated from values of $R^2$ that the relation between mouth coating and storage period (in days) are almost perfect and the graph may be approximated to a straight line.

**Table 4.104:** Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Mouth coating score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 8.1± 0.14a | 7.7± 0.18 | 7.2± 0.19 | 7.0± 0.24 | 6.9± 0.13 | 6.7± 0.16c |
| Swpc$_1$ | 7.9± 0.07b | 7.8± 0.19 | 7.6± 0.29 | 7.4± 0.17 | 7.2± 0.30 | 6.9± 0.13c |
| Swpc$_2$ | 8.5± 0.17ab | 8.1± 0.24 | 7.9± 0.19 | 7.6± 0.14 | 7.2± 0.23 | 7.0± 0.24d |
| Swpc$_3$ | 8.2± 0.13a | 7.8± 0.21 | 7.8± 0.21 | 7.6± 0.19 | 7.4± 0.11 | 7.1± 0.13d |
| Swpc$_4$ | 8.2± 0.17a | 7.9± 0.08 | 7.8± 0.25 | 7.5± 0.27 | 7.3± 0.18 | 7.1± 0.16d |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, Swpc$_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.105:** ANOVA of mouth coating of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 34.15873 | 0.229253 | | |
| Replicate | 4 | 0.557733 | 0.139433 | 3.134321 | 2.46 |
| FA | 4 | 3.833067 | 0.958267 | 21.54087 | 2.46 |
| FB | 5 | 24.03153 | 4.806307 | 108.0409 | 2.3 |
| Comb(A*B) | 29 | 1.534133 | 0.052901 | 1.189164 | 1.63 |
| Error/Res | 107 | 4.76 | 0.044486 | | |
| LSD | 0.264123 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.88:** Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein concentrate

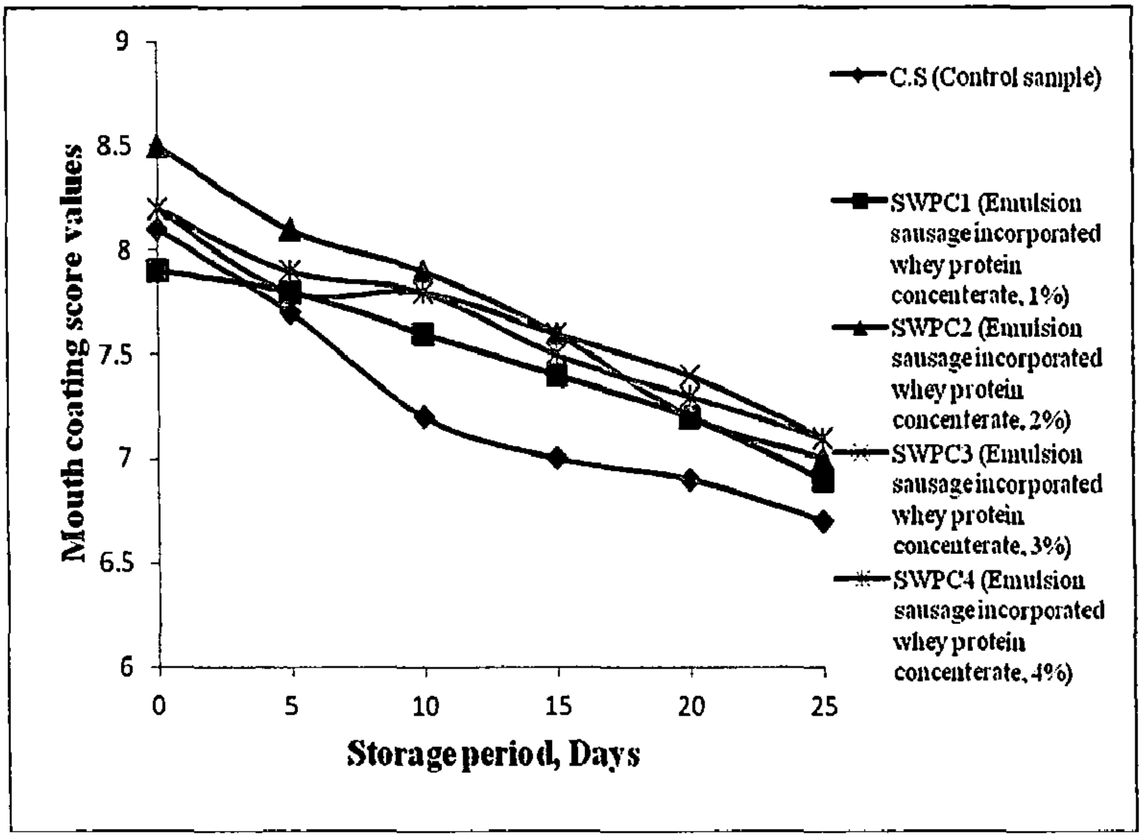



**Fig 4.89:** Regression analysis of mouth coating of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

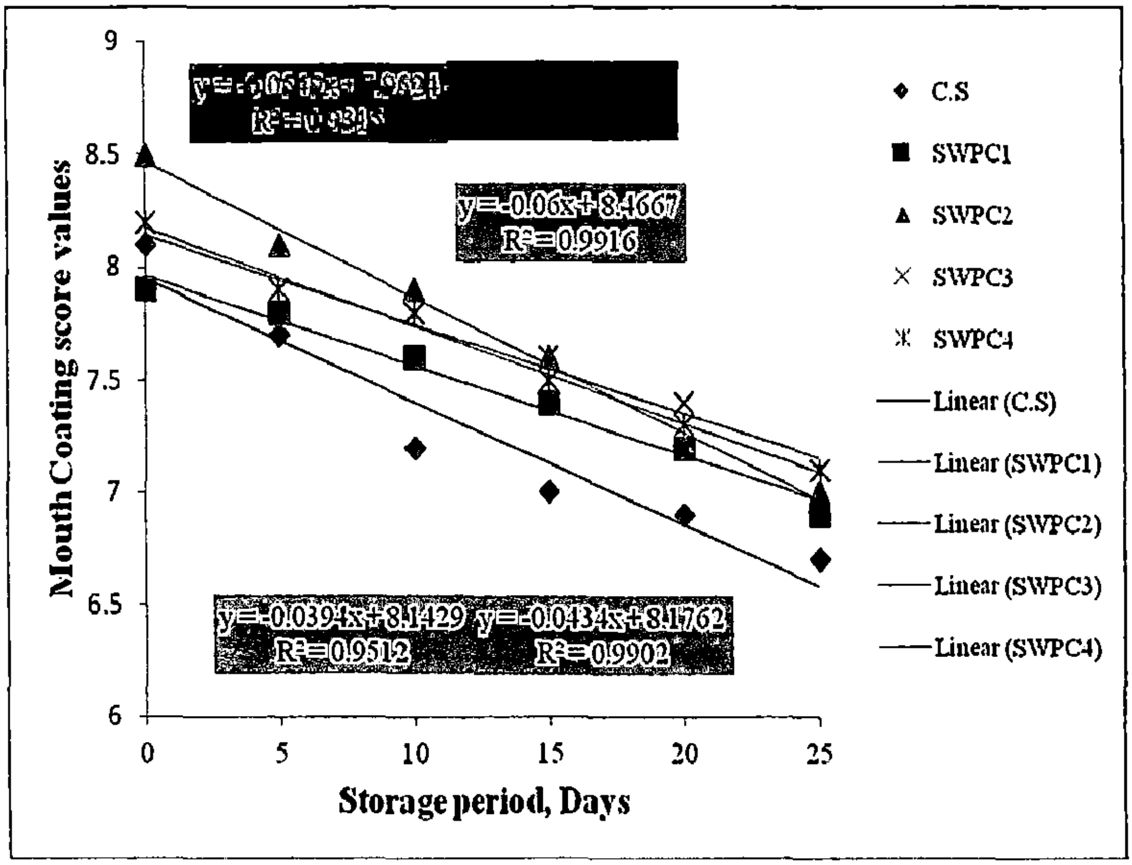


**Table 4.106:** Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Mouth coating score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 8.1± 0.14a | 7.7± 0.18 | 7.2± 0.19 | 7.0± 0.24 | 6.9± 0.13 | 6.7± 0.16c |
| $Swpi_1$ | 8.1± 0.89a | 7.9± 0.08 | 7.6± 0.21 | 7.2± 0.38 | 6.9± 0.05 | 6.8± 0.19c |
| $Swpi_2$ | 8.0± 0.54a | 7.7± 0.16 | 7.5± 0.13 | 7.5± 0.11 | 7.0± 0.11 | 6.7± 0.18c |
| $Swpi_3$ | 7.9± 0.8b | 7.6± 0.20 | 7.5± 0.27 | 7.2± 0.20 | 6.8± 0.1 | 6.6± 0.13c |
| $Swpi_4$ | 8.2± 0.07a | 7.9± 0.11 | 7.7± 0.63 | 7.3± 0.31 | 7.0± 0.35 | 6.8± 0.18c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.107:** ANOVA of mouth coating of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 39.42833 | 0.26462 | | |
| Replicate | 4 | 1.104667 | 0.276167 | 5.15524 | 2.46 |
| FA | 4 | 0.883333 | 0.220833 | 4.122325 | 2.46 |
| FB | 5 | 31.74433 | 6.348867 | 118.5151 | 2.3 |
| Comb(A*B) | 29 | 1.068667 | 0.036851 | 0.687895 | 1.63 |
| Error/Res | 107 | 5.732 | 0.05357 | | |
| LSD | 0.289839 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.90:** Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein isolate

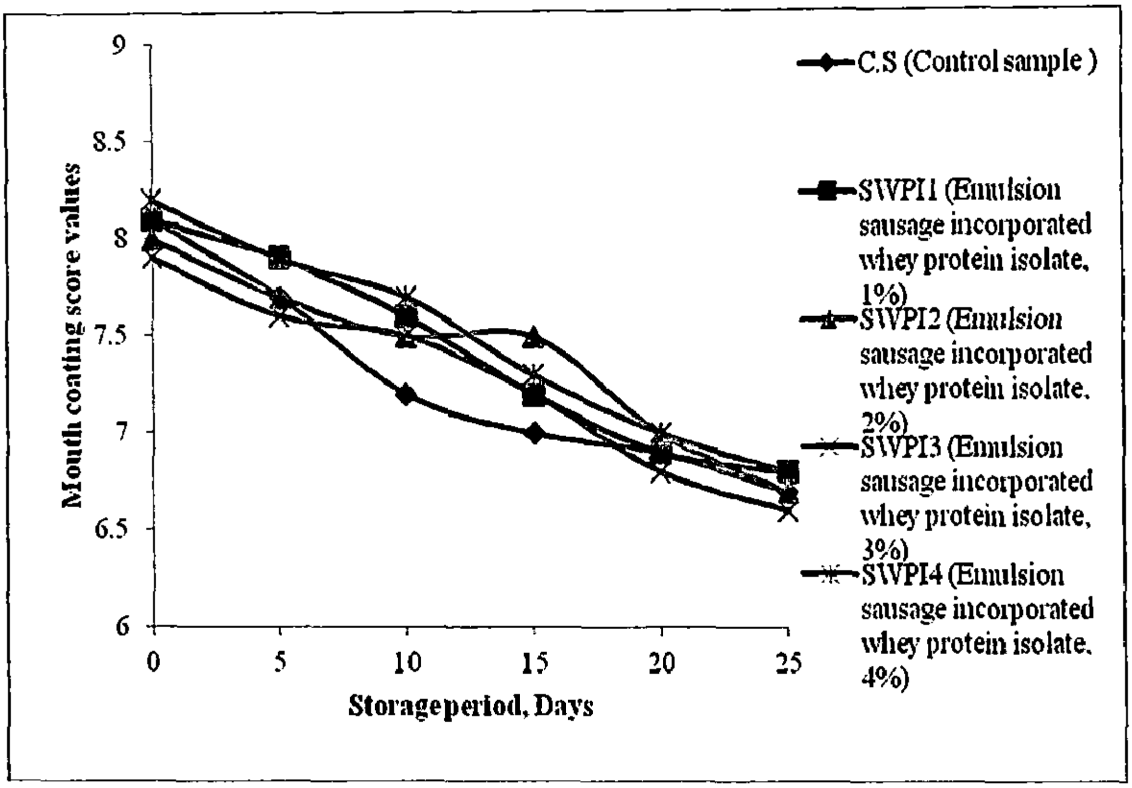



**Fig 4.91:** Regression analysis of mouth coating of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

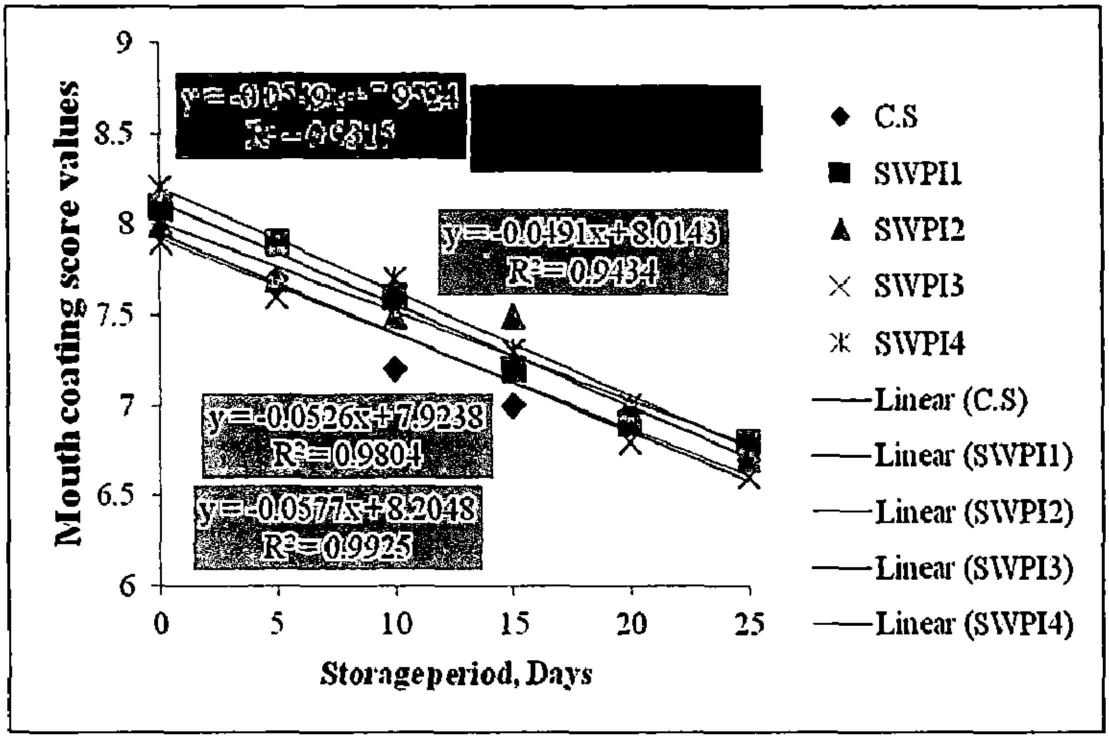


**Table 4.108:** Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Mouth coating score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 8.1± 0.14a | 7.7± 0.18 | 7.2± 0.19 | 7.0± 0.24 | 6.9± 0.13 | 6.7± 0.16c |
| $Swpp_1$ | 7.9± 0.07b | 7.8± 0.07 | 7.5± 0.18 | 7.2± 0.23 | 7.0± 0.13 | 6.6± 0.23c |
| $Swpp_2$ | 7.9± 0.08b | 7.4± 0.20 | 7.2± 0.13 | 7.1± 0.14 | 6.9± 0.13 | 6.5± 0.23cd |
| $Swpp_3$ | 7.9± 0.08b | 7.6± 0.08 | 7.4± 0.27 | 7.1± 0.16 | 6.8± 0.31 | 6.4± 0.28cd |
| $Swpp_4$ | 8.1± 0.08a | 7.9± 0.8 | 7.6± 0.21 | 7.3± 0.23 | 7.1± 0.27 | 6.8± 0.29c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.109:** ANOVA of mouth coating of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 37.1014 | 0.249003 | | |
| Replicate | 4 | 0.951067 | 0.237767 | 5.792585 | 2.46 |
| FA | 4 | 2.1664 | 0.5416 | 13.19472 | 2.46 |
| FB | 5 | 29.5182 | 5.90364 | 143.8273 | 2.3 |
| Comb(A*B) | 29 | 1.0248 | 0.035338 | 0.86092 | 1.63 |
| Error/Res | 107 | 4.392 | 0.041047 | | |
| LSD | 0.253708 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

Fig 4.92: Effect of refrigerated storage (0°C) on mouth coating of emulsion sausages incorporated with different levels of whey protein powder

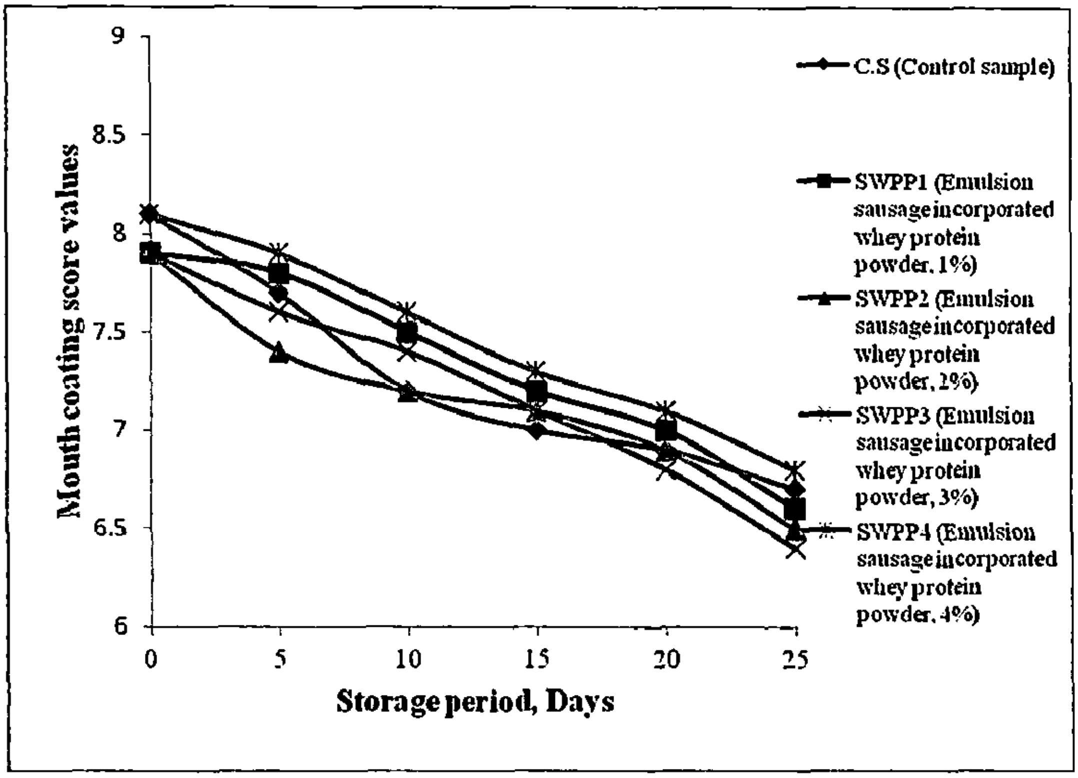
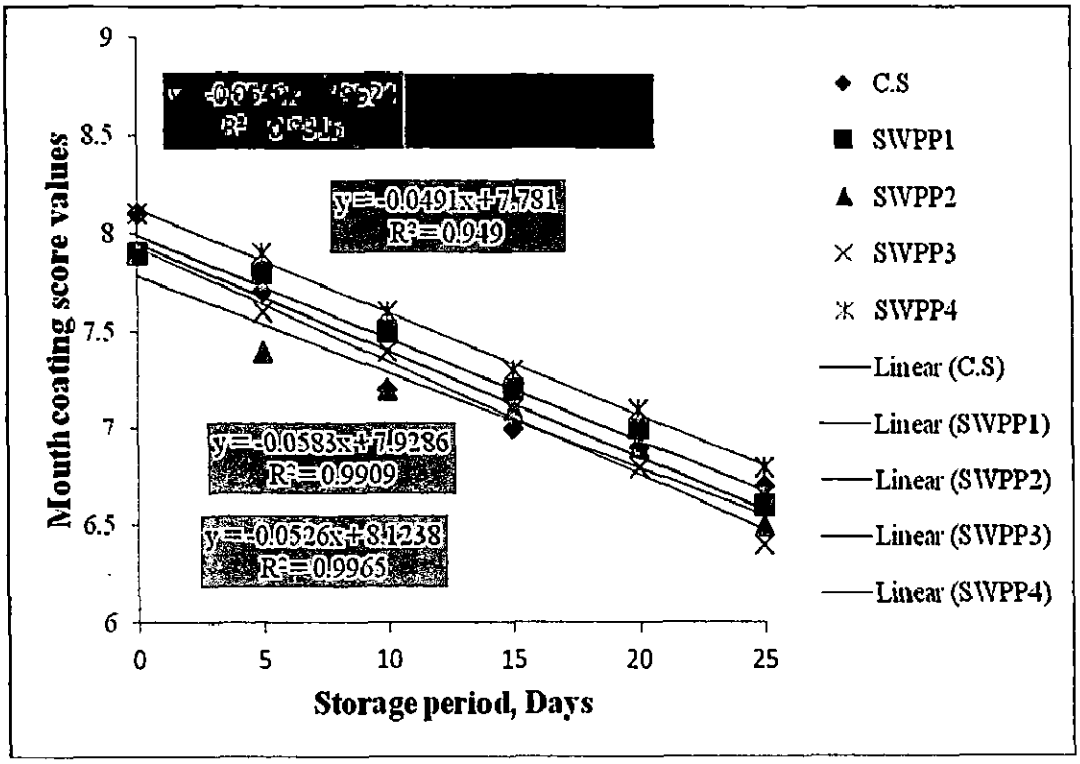



Fig 4.93: Regression analysis of mouth coating of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.6 Juiciness

Tables 4.110, 4.112 and 4.114 present the results of evaluation of juiciness of emulsion sausage samples incorporated with whey protein concentrate, isolate and powder with different levels of 1, 2, 3 and 4% during refrigerated storage. All sausage samples treated with whey protein concentrate isolate and whey protein powder with levels (1, 2, 3 and 4%) had score values of juiciness ranging between 7-9. It represented conditions between like moderately to like extremely. Different levels of whey protein isolate and powder did not significantly ($p<0.05$) affect the juiciness score values of fresh condition of emulsion sausages. But whey protein concentrate significantly ($p<0.05$) increase score value of juiciness of the emulsion sausages samples. Interaction between whey protein products and storage period did not significantly ($p<0.05$) affect the juiciness score values (Tables 4.111, 4.113 and 4.115). During refrigerated storage (0°C) the score values of juiciness decreased due to the loss of moisture content (Figures 4.94, 4.96 and 4.98). The score values of juiciness of all samples on $25^{th}$ day of storage were found to be in range of 6.6–7.1.

Figures (4.95, 4.97 and 4.99) represented the linear regression of juiciness score values of emulsion sausages incorporated with whey protein concentrate, isolate and powder at the levels of (1, 2, 3 and 4%) during refrigerated storage period. The equation of regression line and correlation coefficients are shown on the regression graph. The negative sign in the coefficients of explain that there was consistent decrease of juiciness score values during storage period. The values of $R^2$ for all samples of emulsion sausages incorporated with whey protein products with different levels of 1, 2, 3 and 4% were between 0.9325–0.997, which shows relation between juiciness of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

**Table 4.110:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Juiciness score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.7± 0.14a | 7.5± 0.35 | 7.3± 0.15 | 6.8± 0.11 | 6.6± 0.16 | 6.6± 0.14c |
| **$Swpc_1$** | 8.3± 0.10b | 8.0± 0.13 | 8.0± 0.35 | 7.8± 0.18 | 7.5± 0.37 | 7.1± 0.18d |
| **$Swpc_2$** | 8.7± 0.89bc | 8.1± 0.21 | 7.7± 0.20 | 7.5± 0.23 | 7.0± 0.25 | 6.7± 0.46c |
| **$Swpc_3$** | 8.3± 0.13b | 7.9± 0.13 | 7.6± 0.39 | 7.2± 0.21 | 6.9± 0.21 | 6.5± 0.13c |
| **$Swpc_4$** | 8.2± 0.44b | 7.8± 0.26 | 7.6± 0.37 | 7.2± 0.26 | 7.0± 0.19 | 6.8± 0.18c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.111:** ANOVA of juiciness of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 56.67893 | 0.380396 | | |
| **Replicate** | 4 | 0.290267 | 0.072567 | 1.034181 | 2.46 |
| **FA** | 4 | 9.226933 | 2.306733 | 32.87433 | 2.46 |
| **FB** | 5 | 37.77173 | 7.554347 | 107.6605 | 2.3 |
| **Comb(A*B)** | 29 | 2.172267 | 0.074906 | 1.067517 | 1.63 |
| **Error/Res** | 107 | 7.508 | 0.070168 | | |
| **LSD** | 0.331715 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.94:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein concentrate

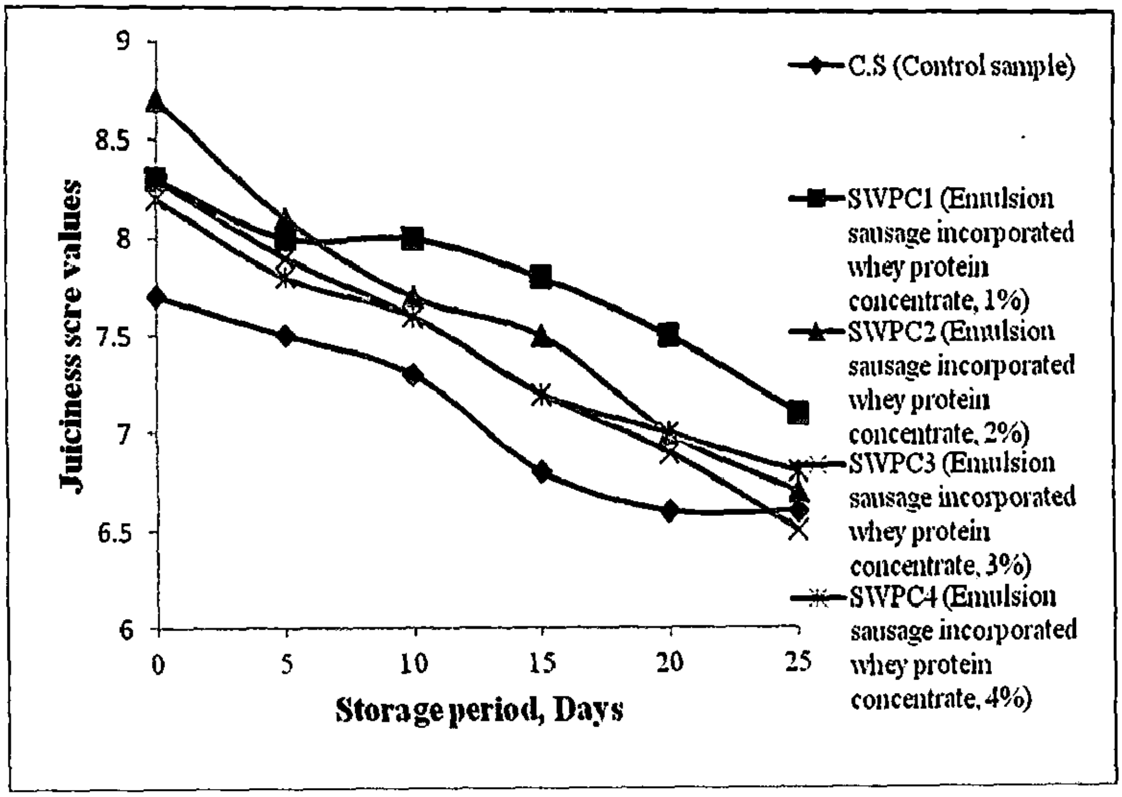



**Fig 4.95:** Regression analysis of juiciness of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

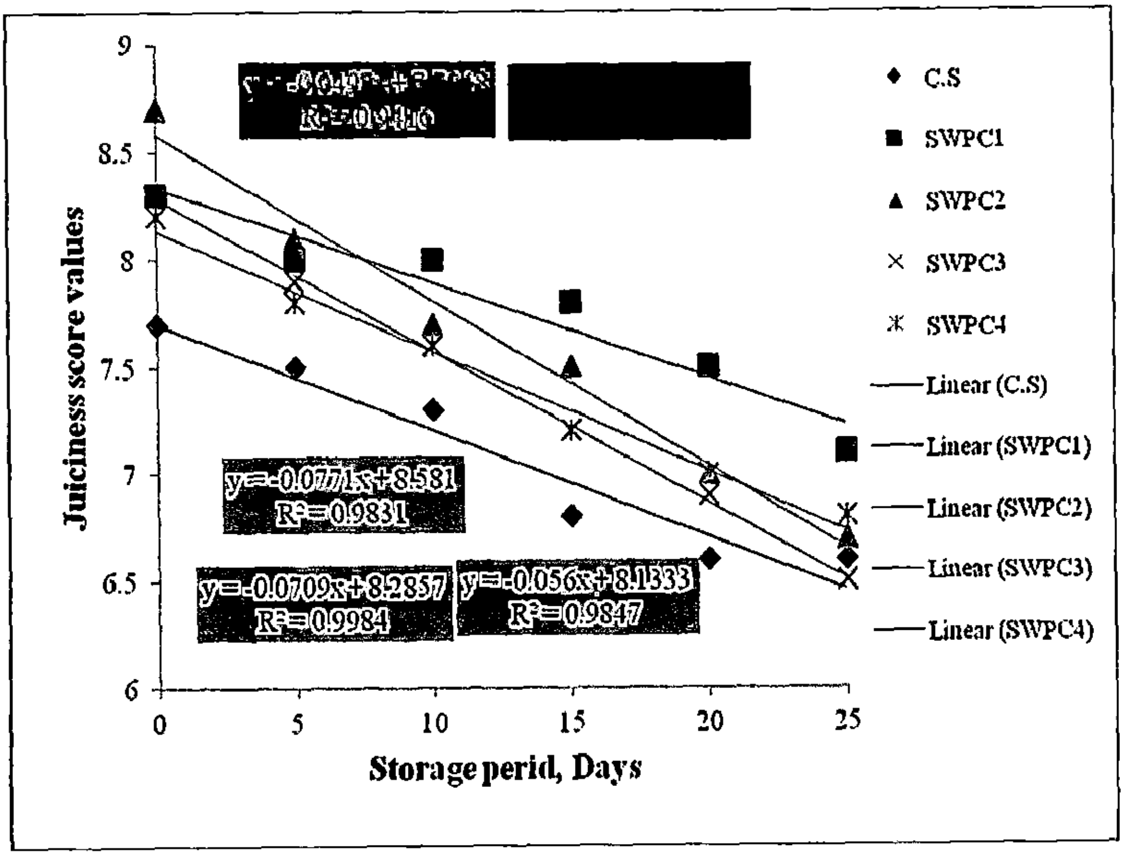


**Table 4.112:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Juiciness score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.7± 0.14a | 7.5± 0.35 | 7.3± 0.15 | 6.8± 0.11 | 6.6± 0.16 | 6.6± 0.14c |
| **$Swpi_1$** | 7.9± 0.07a | 7.9± 0.07 | 7.7± 0.23 | 7.3± 0.24 | 7.1± 0.17 | 6.8± 0.08c |
| **$Swpi_2$** | 7.6± 0.08a | 7.6± 0.08 | 7.5± 0.36 | 7.4± 0.24 | 7.2± 0.23 | 6.9± 0.30c |
| **$Swpi_3$** | 8.0± 0.08b | 7.7± 0.15 | 7.5± 0.35 | 7.2± 0.23 | 7.0± 0.18 | 6.7± 0.11c |
| **$Swpi_4$** | 7.8± 0.05a | 7.5± 0.17 | 7.1± 0.16 | 7.0± 0.35 | 6.9± 0.14 | 6.6± 0.15c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.113:** ANOVA of juiciness of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 30.42773 | 0.204213 | | |
| **Replicate** | 4 | 0.399067 | 0.099767 | 2.101385 | 2.46 |
| **FA** | 4 | 3.183067 | 0.795767 | 16.76123 | 2.46 |
| **FB** | 5 | 20.64933 | 4.129867 | 86.98735 | 2.3 |
| **Comb(A*B)** | 29 | 1.515333 | 0.052253 | 1.100602 | 1.63 |
| **Error/Res** | 107 | 5.08 | 0.047477 | | |
| **LSD** | 0.272857 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square,
LSD = Least significance difference, FA = Different levels of whey protein incorporated,
FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.96:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein isolate

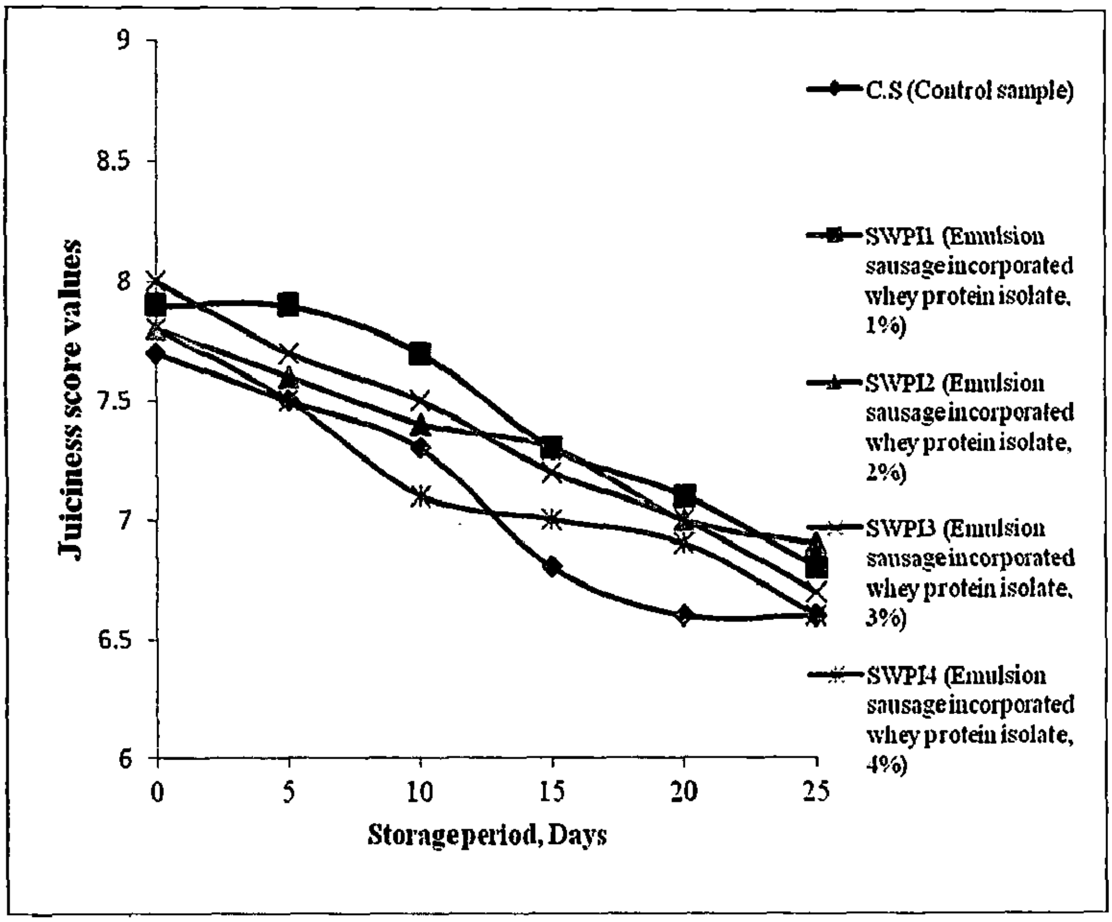



**Fig 4.97:** Regression analysis of juiciness of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

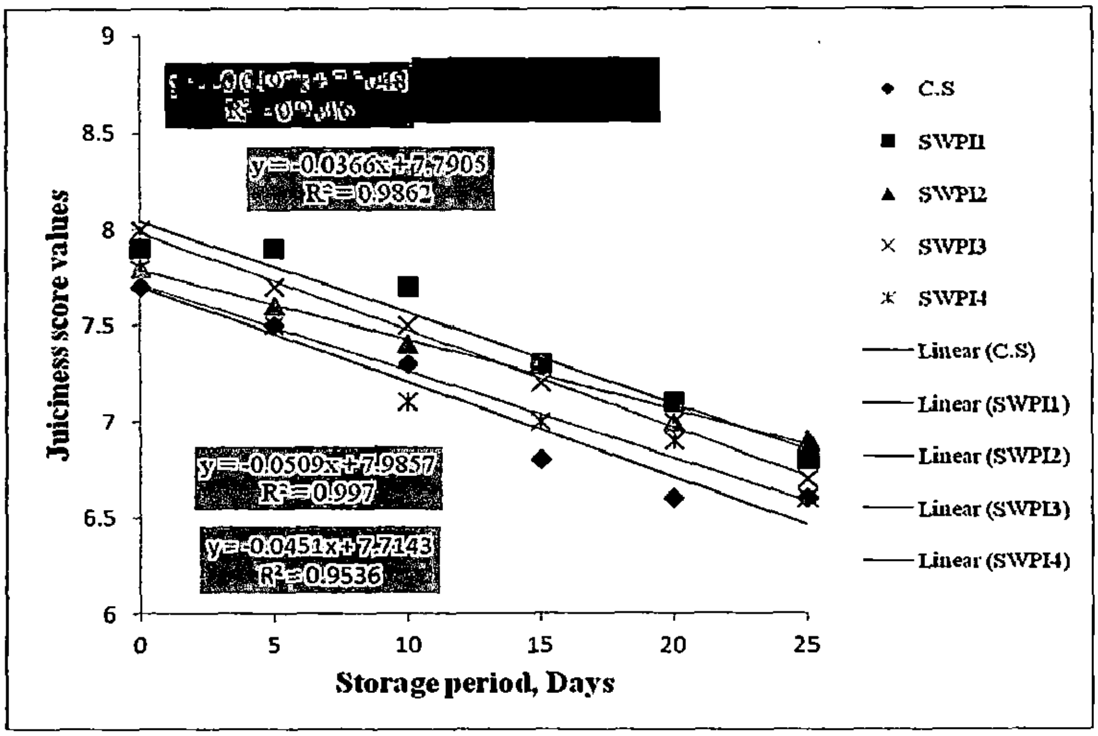


**Table 4.114:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Juiciness score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.7± 0.14a | 7.5± 0.35 | 7.3± 0.15 | 6.8± 0.11 | 6.6± 0.16 | 6.6± 0.14c |
| **$Swpp_1$** | 8.1± 0.08b | 8.0± 0.10 | 7.6± 0.14 | 7.4± 0.21 | 7.1± 0.15 | 6.8± 0.13cd |
| **$Swpp_2$** | 7.9± 0.05a | 7.8± 0.13 | 7.5± 0.35 | 7.3± 0.37 | 7.0± 0.33 | 6.9± 0.16cd |
| **$Swpp_3$** | 8.0± 0.05b | 7.7± 0.21 | 7.6± 0.25 | 7.3± 0.23 | 6.9± 0.35 | 6.6± 0.17c |
| **$Swpp_4$** | 8.0± 0.89b | 7.8± 0.15 | 7.5± 0.32 | 7.2± 0.16 | 7.0± 0.22 | 6.8± 0.18cd |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.115:** ANOVA of juiciness of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 37.92773 | 0.254549 | | |
| **Replicate** | 4 | 0.555067 | 0.138767 | 2.703575 | 2.46 |
| **FA** | 4 | 3.401067 | 0.850267 | 16.56565 | 2.46 |
| **FB** | 5 | 28.33413 | 5.666827 | 110.4061 | 2.3 |
| **Comb(A*B)** | 29 | 0.700533 | 0.024156 | 0.470635 | 1.63 |
| **Error/Res** | 107 | 5.492 | 0.051327 | | |
| **LSD** | 0.283706 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.98:** Effect of refrigerated storage (0°C) on juiciness of emulsion sausages incorporated with different levels of whey protein powder

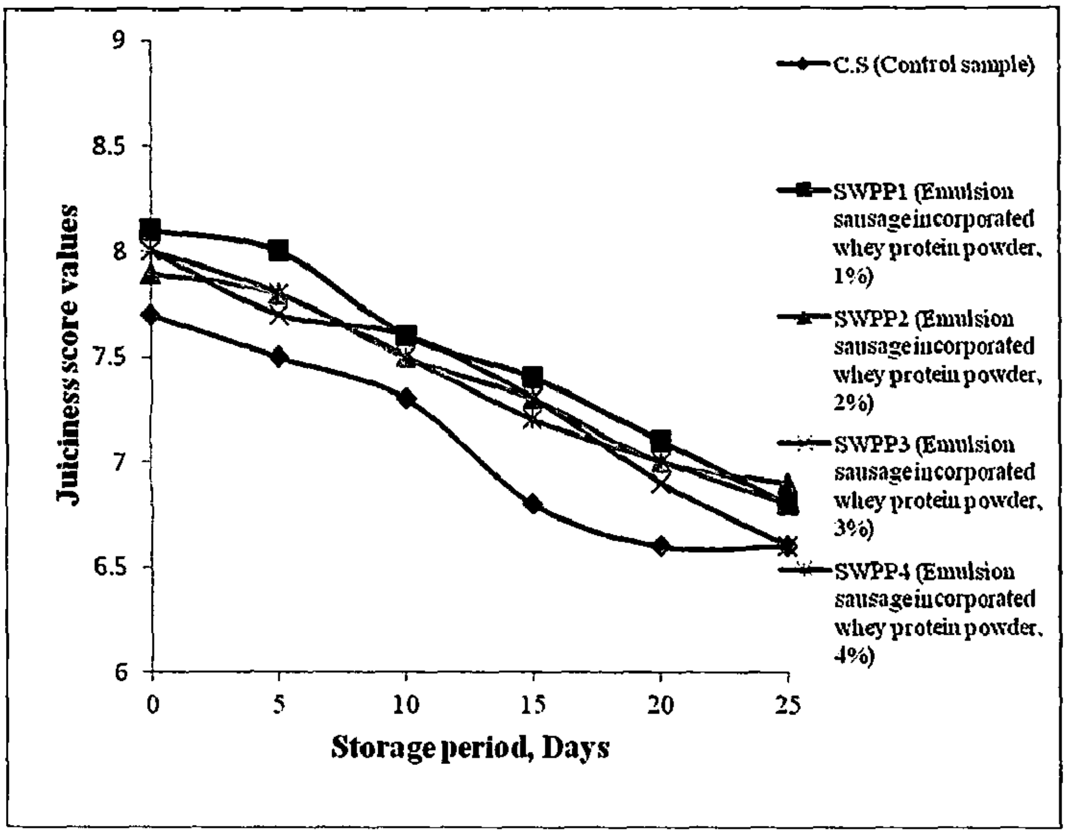
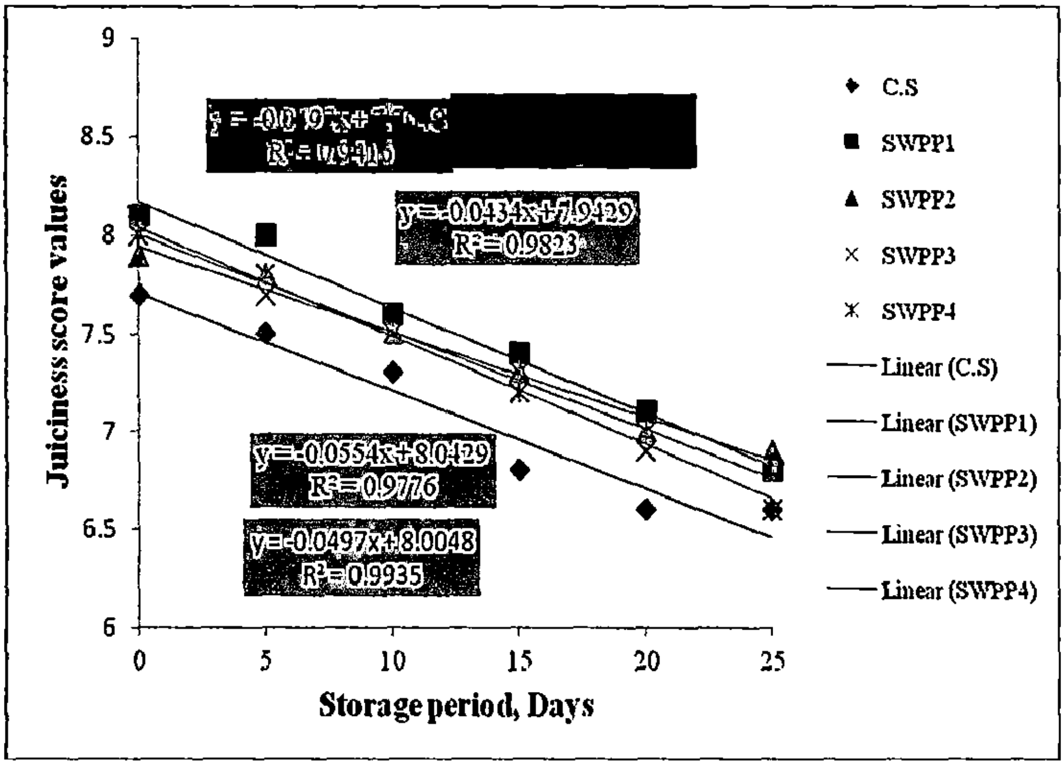



**Fig 4.99:** Regression analysis of juiciness of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.4.7 Palatability

Tables 4.116, 4.118 and 4.120 present the results of sensory evaluation (palatability) of emulsion sausages samples with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) during refrigerated storage. All the emulsion sausage samples in fresh condition had the score values of palatability between 7.8 to 8.5. It represented condition between liked very much and liked extremely. The score value of palatability of control sample was 7.2. Different levels of whey protein concentrate, isolate and whey protein powder did not significantly ($p<0.05$) affect score values of palatability of emulsion sausages in fresh condition. The interaction between whey protein products and storage period significantly ($p<0.05$) decreased the palatability score values of emulsion sausages (ANOVA Table 4.117, 119 and 121). During refrigerated storage at $0^{\circ}C$ the score values of palatability of controlled and treated emulsion sausages decreased (Figure 4.100, 4.102, 4.104). The mean score values of palatability on 25th day of storage were between of 6.6-6.8 for all samples. Kala *et al.*, (2007) reported the palatability of chicken emulsion patties during different intervals of refrigerated storage was highly acceptable with score approaching 'very good' rating.

Figures (4.101, 4.103 and 4.105) represent the linear regression of palatability score values of emulsion sausages incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) during storage period. The equation of regression line and correlation coefficients were shown on the regression graph. The negative sign in the coefficients of x explains that there was constant decrease of palatability score values during storage period. The values of $R^2$ for all sausages samples produced with different levels (1, 2, 3 and 4%) whey protein products were in the range of 0.9502-0.999. The values of $R^2$ exhibits that a linear system follows for the relation between palatability score values and storage period, and graph may be approximately represented by a straight line.

**Table 4.116:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Palatability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.2± 0.17a | 7.1± 0.36 | 6.8± 0.13 | 6.6± 0.42 | 6.3± 0.20 | 6.2± 0.18c |
| $Swpc_1$ | 7.8± 0.21ab | 7.7± 0.27 | 7.5± 0.31 | 7.4± 0.39 | 7.1± 0.16 | 6.8± 0.23cd |
| $Swpc_2$ | 8.3± 0.26b | 8.2± 0.25 | 8.0± 0.38 | 7.6± 0.41 | 7.3± 0.22 | 6.8± 0.15cd |
| $Swpc_3$ | 8.2± 0.20b | 8.0± 0.17 | 7.7± 0.19 | 7.3± 0.26 | 6.9± 0.32 | 6.6± 0.16cd |
| $Swpc_4$ | 8.2± 0.17b | 7.8± 0.20 | 7.5± 0.36 | 7.4± 0.42 | 6.8± 0.34 | 6.4± 0.42c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.117:** ANOVA of palatability of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 63.01573 | 0.422924 | | |
| Replicate | 4 | 0.4964 | 0.1241 | 1.341283 | 2.46 |
| FA | 4 | 16.62173 | 4.155433 | 44.91226 | 2.46 |
| FB | 5 | 34.53333 | 6.906667 | 74.64781 | 2.3 |
| Comb(A*B) | 29 | 1.960667 | 0.067609 | 0.730726 | 1.63 |
| Error/Res | 107 | 9.9 | 0.092523 | | |
| LSD | 0.380909 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.100:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein concentrate

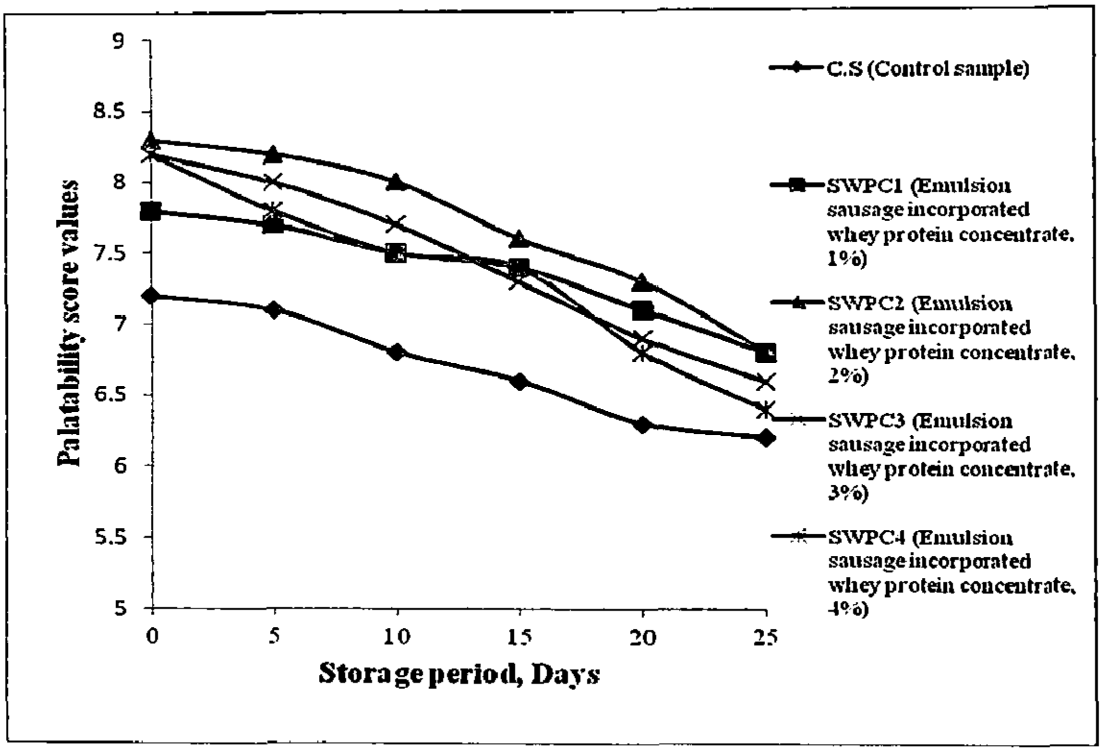



**Fig 4.101:** Regression analysis of palatability of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

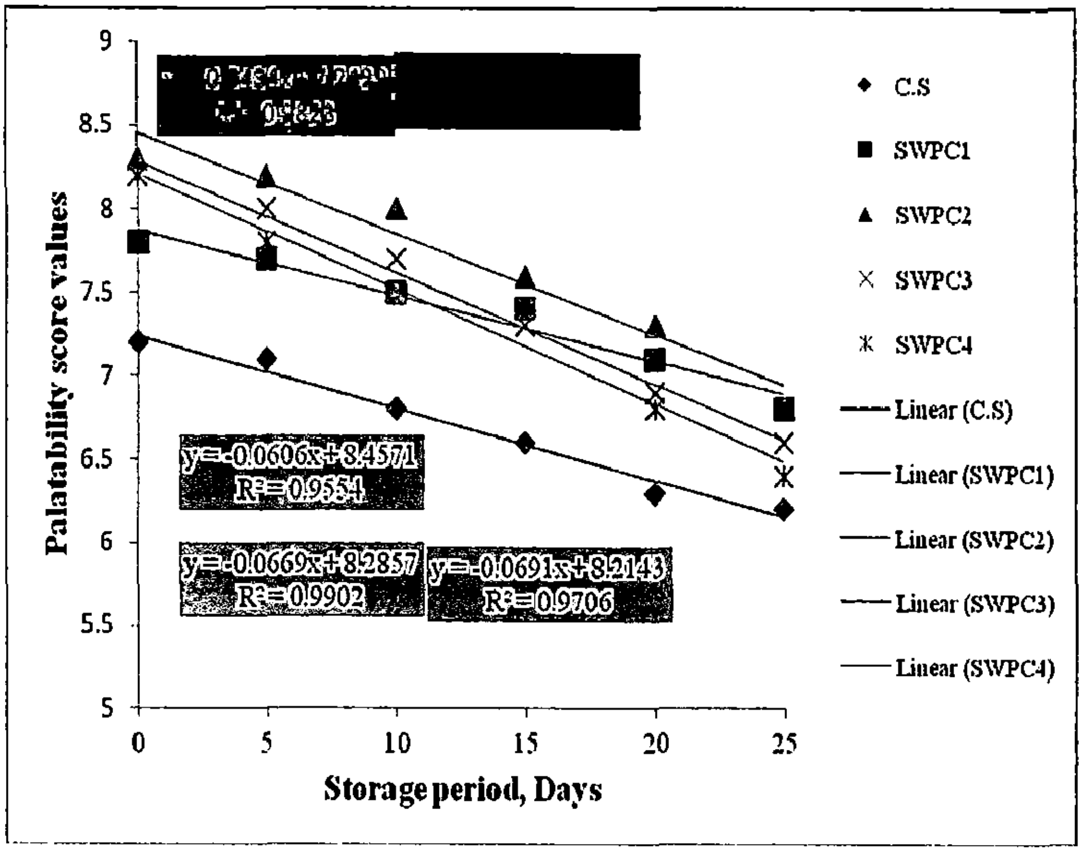


**Table 4.118:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Palatability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.2± 0.17a | 7.1± 0.36 | 6.8± 0.13 | 6.6± 0.42 | 6.3± 0.20 | 6.2± 0.18c |
| **$Swpi_1$** | 8.5± 0.07b | 8.4± 0.19 | 8.1± 0.21 | 7.7± 0.25 | 7.1± 0.21 | 6.6± 0.28c |
| **$Swpi_2$** | 8.2±b 0.07 | 7.8± 0.50 | 7.5± 0.38 | 7.2± 0.21 | 6.9± 0.10 | 6.4± 0.31c |
| **$Swpi_3$** | 8.2± 0.08b | 8.1± 0.03 | 7.8± 0.14 | 7.5± 0.32 | 7.1± 0.27 | 6.5± 0.39c |
| **$Swpi_4$** | 8.3± 0.08b | 8.1± 0.13 | 7.7± 0.27 | 7.3± 0.20 | 6.9± 0.45 | 6.5± 0.53c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1, 2, 3, 4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.119:** ANOVA of palatability of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 77.0806 | 0.517319 | | |
| **Replicate** | 4 | 1.666933 | 0.416733 | 4.741649 | 2.46 |
| **FA** | 4 | 17.7596 | 4.4399 | 50.51779 | 2.46 |
| **FB** | 5 | 47.7502 | 9.55004 | 108.6617 | 2.3 |
| **Comb(A*B)** | 29 | 2.1668 | 0.074717 | 0.850143 | 1.63 |
| **Error/Res** | 107 | 9.404 | 0.087888 | | |
| **LSD** | 0.371244 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.102:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein isolate

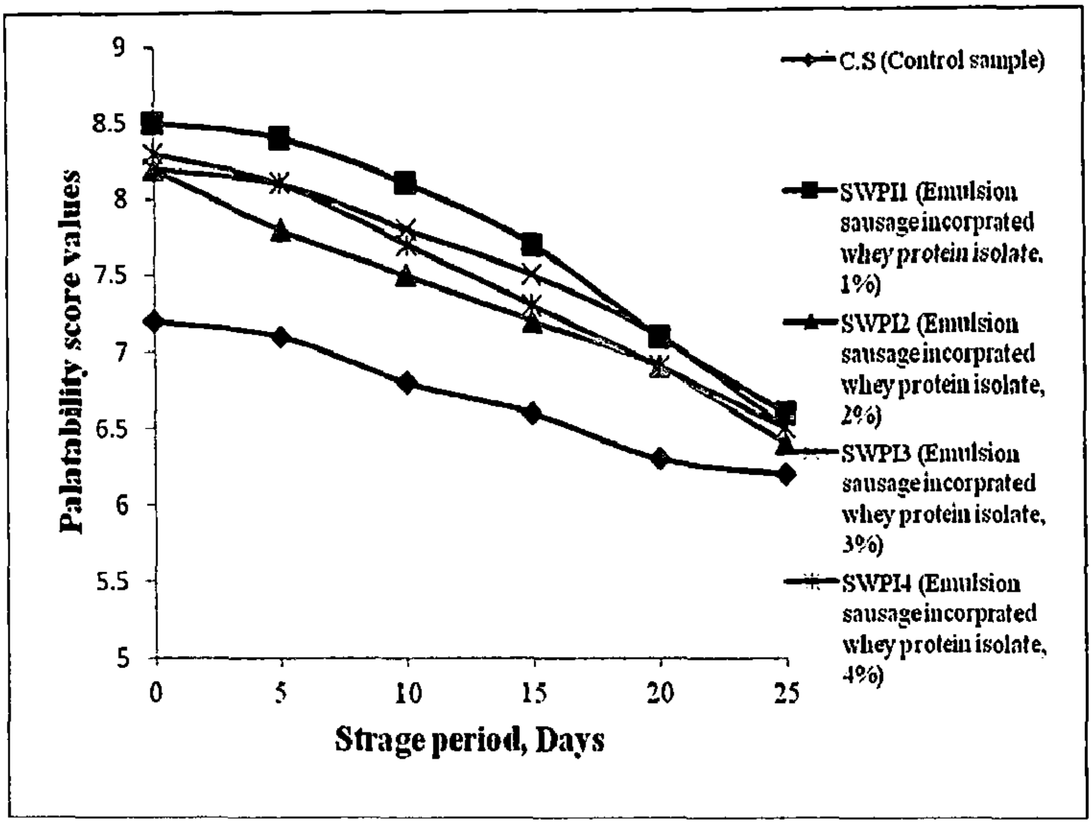



**Fig 4.103:** Regression analysis of palatability of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

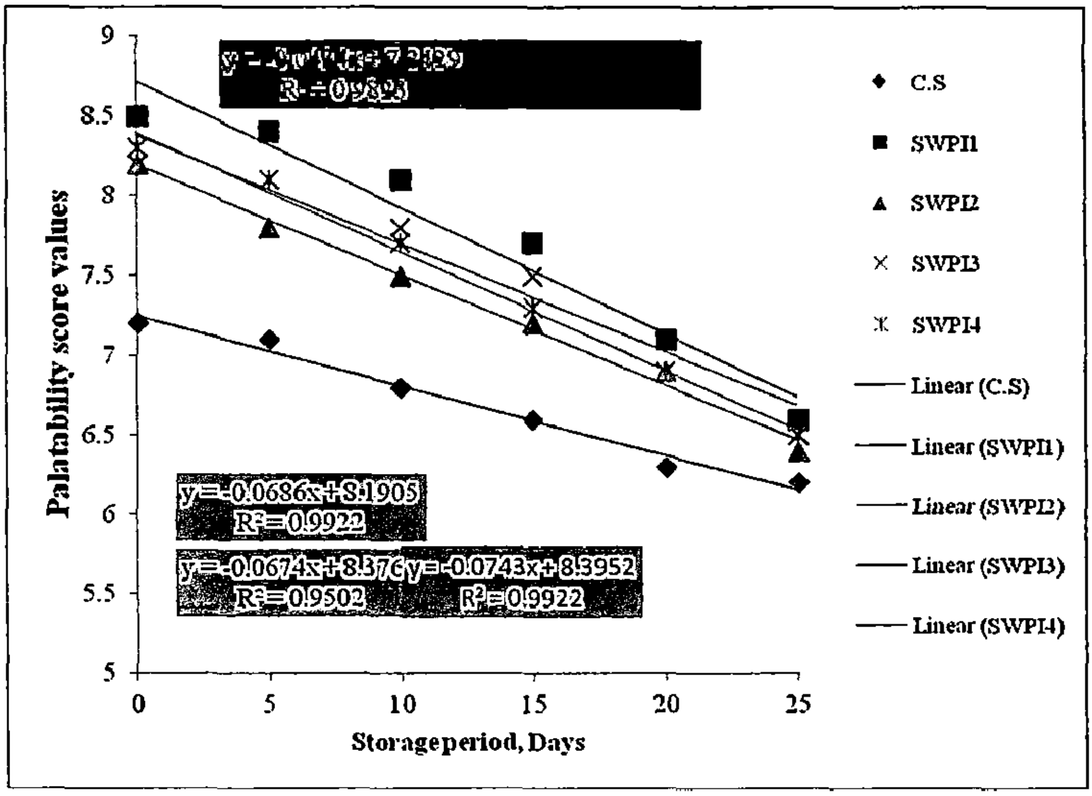


**Table 4.120:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Palatability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.2± 0.17a | 7.1± 0.36 | 6.8± 0.13 | 6.6± 0.42 | 6.3± 0.20 | 6.2± 0.18c |
| $Swpp_1$ | 8.5± 0.02b | 8.1± 0.16 | 7.6± 0.25 | 7.2± 0.25 | 6.7± 0.21 | 6.3± 0.24c |
| $Swpp_2$ | 8.6± 0.01b | 8.2± 0.21 | 7.6± 0.42 | 7.3± 0.32 | 6.9± 0.53 | 6.3± 0.49c |
| $Swpp_3$ | 8.4± 0.02b | 8.1± 0.19 | 7.8± 0.19 | 7.2± 0.28 | 6.8± 0.27 | 6.4± 0.29c |
| $Swpp_4$ | 8.3± 0.05b | 7.8± 0.10 | 7.3± 0.10 | 6.8± 0.29 | 6.4± 0.28 | 6.2± 0.14c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.121:** ANOVA of palatability of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 92.93619 | 0.623733 | | |
| Replicate | 4 | 2.366224 | 0.591556 | 7.026354 | 2.46 |
| FA | 4 | 12.3992 | 3.099799 | 36.81864 | 2.46 |
| FB | 5 | 67.56312 | 13.51262 | 160.4996 | 2.3 |
| Comb(A*B) | 29 | 3.965427 | 0.136739 | 1.62415 | 1.63 |
| Error/Res | 107 | 9.00844 | 0.084191 | | |
| LSD | 0.363352 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.104:** Effect of refrigerated storage (0°C) on palatability of emulsion sausages incorporated with different levels of whey protein powder

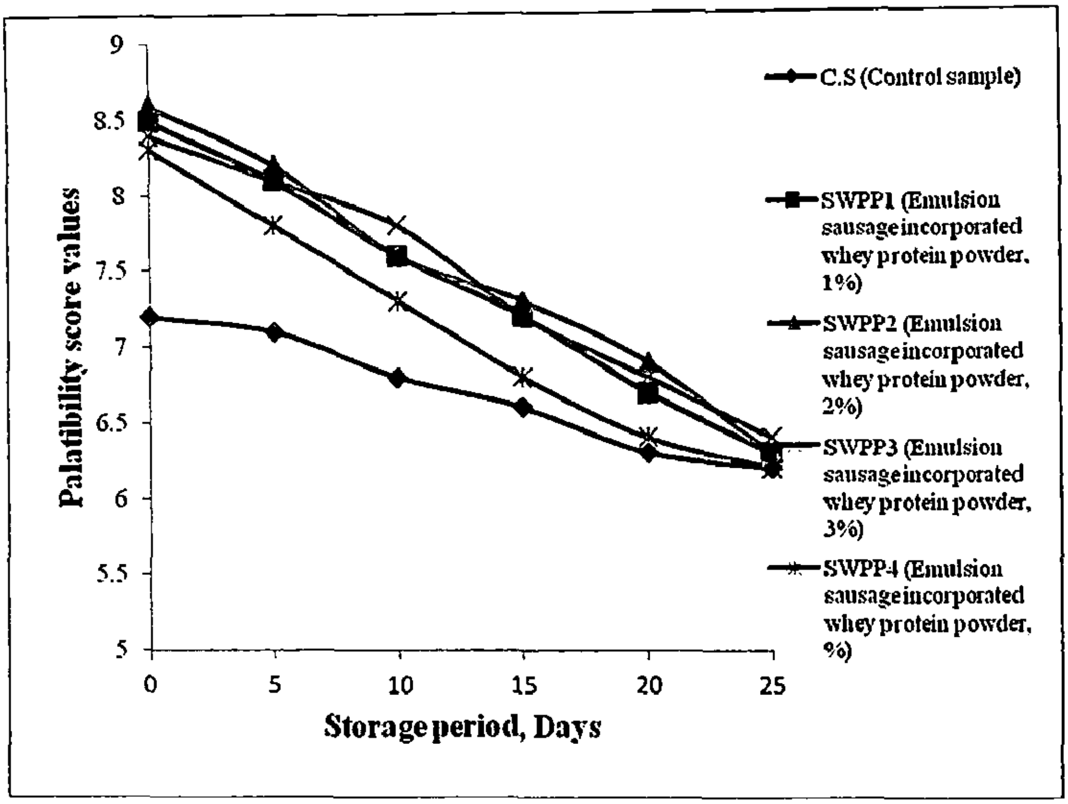
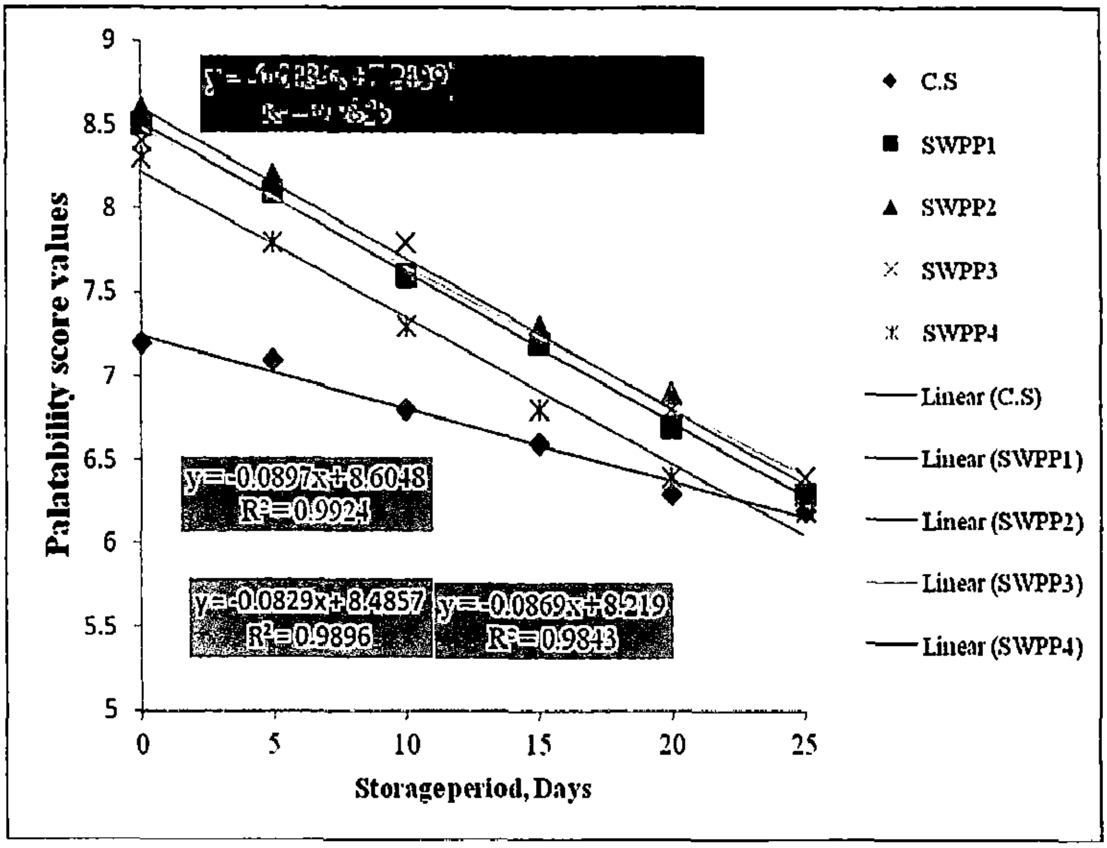



**Fig 4.105:** Regression analysis of palatability of emulsion sausages incorporated with *different levels of whey protein powder during refrigerated storage (0°C)*



#### 4.2.4.8 Overall acceptability

Tables 4.122, 4.124 and 4.126 present the results of sensory evaluation for overall acceptability of emulsion sausages treated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) during refrigerated storage. All the emulsion sausage samples treated with whey protein concentrate isolate and whey protein powder with different levels (1, 2, 3 and 4%) had the score values of overall acceptability between 8 to 9. It represented condition between like very much to like extremely. Different levels of whey protein products significantly ($p<0.05$) increased the overall acceptability score values of fresh condition of emulsion sausages samples. Interaction between whey protein products and storage period significantly ($p<0.05$) decreased the overall acceptability score values. During refrigerated storage (0°C) the score values of overall acceptability significantly ($p<0.05$) decreased due to the decrease of other sensory score values during refrigerated storage (ANOVA Tables 4.123, 4.125 and 4.127). However the score values of overall acceptability on 25$^{th}$ day of storage were between of 6.6–7.7 for all samples. Figures (4.106, 4.108 and 4.109) represent the decreasing profile of overall acceptability score values of controlled and treated emulsion sausages.

Figures (4.107, 4.109 and 4.111) represented the linear regression of score values for overall acceptability of emulsion sausage samples incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) during refrigerated storage period. The equation of regression line and correlation coefficients were shown on the regression graph. The negative sign in the coefficients of explains that there was constant decrease of overall acceptability score values during storage period. The values of $R^2$ for all samples of emulsion sausage samples treated with whey protein products with different levels (1, 2, 3 and 4%) were between 0.9161–0.9967, which shows relation between acceptability of the samples and storage period were almost perfect and the graph may be approximated to a straight line.

**Table 4.122:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein concentrate

| Sample Code | Overall acceptability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.5± 0.28a | 7.5± 0.21 | 7.4± 0.28 | 7.1± 0.22 | 6.9± 0.63 | 6.8± 0.72c |
| $Swpc_1$ | 8.1± 0.22b | 7.9± 0.11 | 7.7± 0.29 | 7.6± 0.36 | 7.3± 0.33 | 7.1± 0.20d |
| $Swpc_2$ | 8.5± 0.24b | 8.5± 0.14 | 8.2± 0.17 | 8.0± 0.35 | 7.9± 0.60 | 7.7± 0.49ef |
| $Swpc_3$ | 8.4± 0.26b | 8.2± 0.14 | 8.0± 0.35 | 7.8± 0.28 | 7.6± 0.37 | 7.3± 0.23d |
| $Swpc_4$ | 8.0± 0.50b | 7.7± 0.14 | 7.4± 0.21 | 7.1± 0.53 | 6.8± 0.73 | 6.6± 0.41c |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.123:** ANOVA of overall acceptability of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 55.4344 | 0.372043 | | |
| Replicate | 4 | 1.6784 | 0.4196 | 2.604246 | 2.46 |
| FA | 4 | 18.30707 | 4.576767 | 28.40569 | 2.46 |
| FB | 5 | 18.6424 | 3.72848 | 23.1408 | 2.3 |
| Comb(A*B) | 29 | 1.244933 | 0.042929 | 0.266437 | 1.63 |
| Error/Res | 107 | 17.24 | 0.161121 | | |
| LSD | 0.502657 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.106:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein concentrate

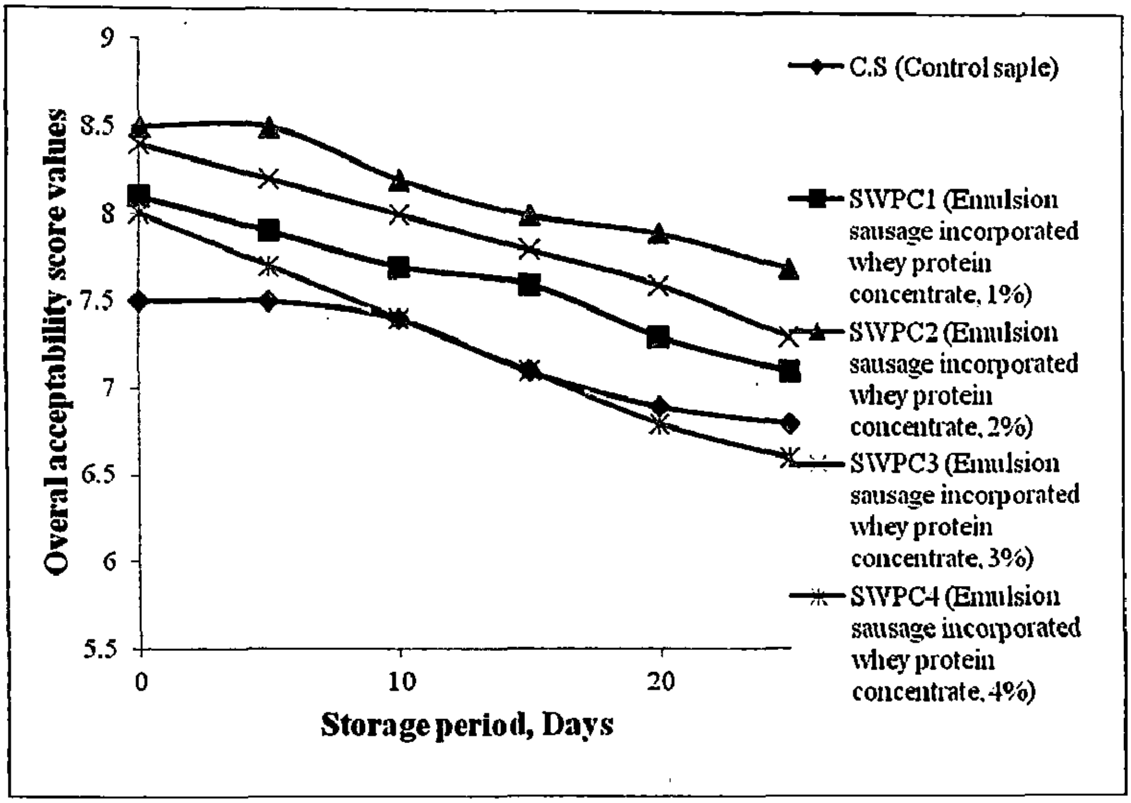



**Fig 4.107:** Regression analysis of overall acceptability of emulsion sausages incorporated with different levels of whey protein concentrate during refrigerated storage (0°C)

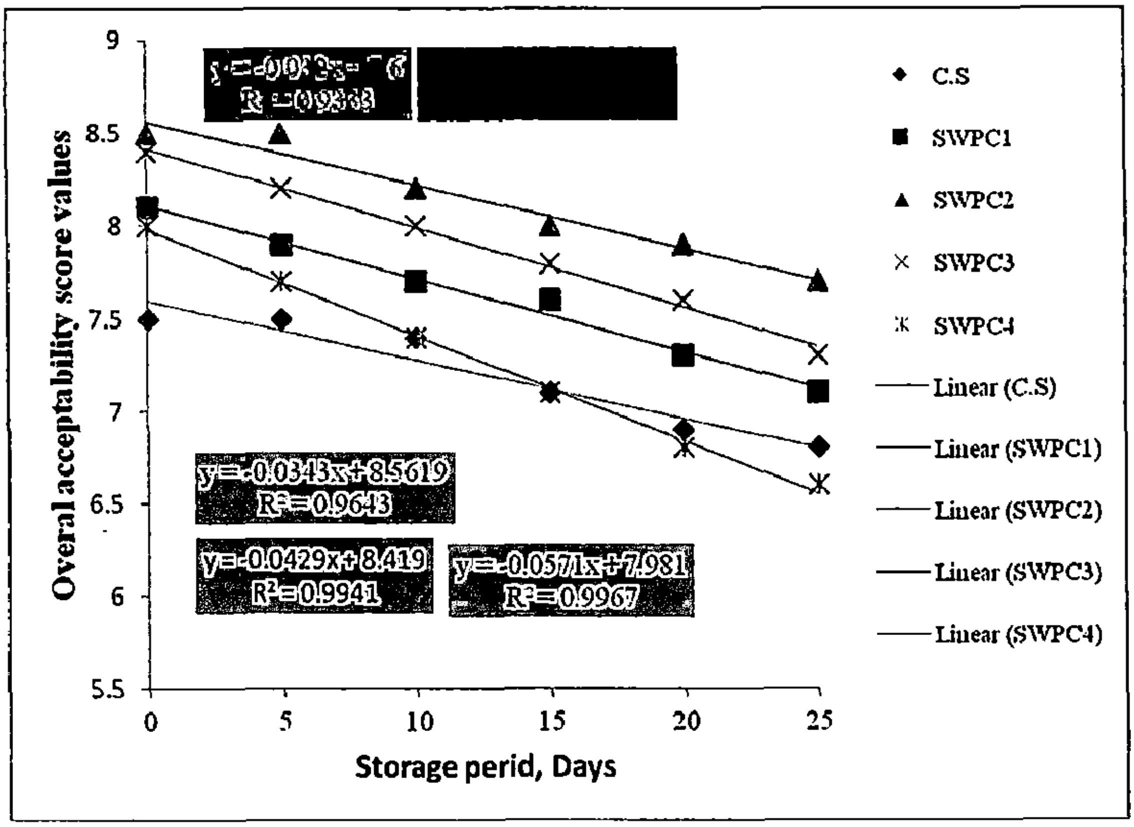


**Table 4.124:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein isolate

| Sample Code | Overall acceptability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| **Cs** | 7.5± 0.28a | 7.5± 0.21 | 7.4± 0.28 | 7.1± 0.22 | 6.9± 0.63 | 6.8± 0.72d |
| **$Swpi_1$** | 8.0± 0.04b | 8.0± 0.35 | 7.9± 0.63 | 7.8± 0.41 | 7.6± 0.40 | 7.3± 0.41e |
| **$Swpi_2$** | 8.0± 0.05b | 8.0± 0.28 | 7.8± 0.56 | 7.8± 0.55 | 7.5± 0.45 | 7.2± 0.43e |
| **$Swpi_3$** | 8.1± 0.05b | 8.0± 0.70 | 7.9± 0.24 | 7.6± 0.38 | 7.5± 0.35 | 7.4± 0.35e |
| **$Swpi_4$** | 7.9± 0.08c | 7.8± 0.56 | 7.6± 0.54 | 7.5± 0.51 | 7.3± 0.40 | 7.1± 0.47e |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

**Table 4.125:** ANOVA of overall acceptability of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| **Total** | 149 | 39.3616 | 0.264172 | | |
| **Replicate** | 4 | 2.012267 | 0.503067 | 2.386845 | 2.46 |
| **FA** | 4 | 6.7216 | 1.6804 | 7.97281 | 2.46 |
| **FB** | 5 | 9.628 | 1.9256 | 9.136183 | 2.3 |
| **Comb(A*B)** | 29 | 0.46 | 0.015862 | 0.075259 | 1.63 |
| **Error/Res** | 107 | 22.552 | 0.210766 | | |
| **LSD** | 0.574905 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.108:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein isolate

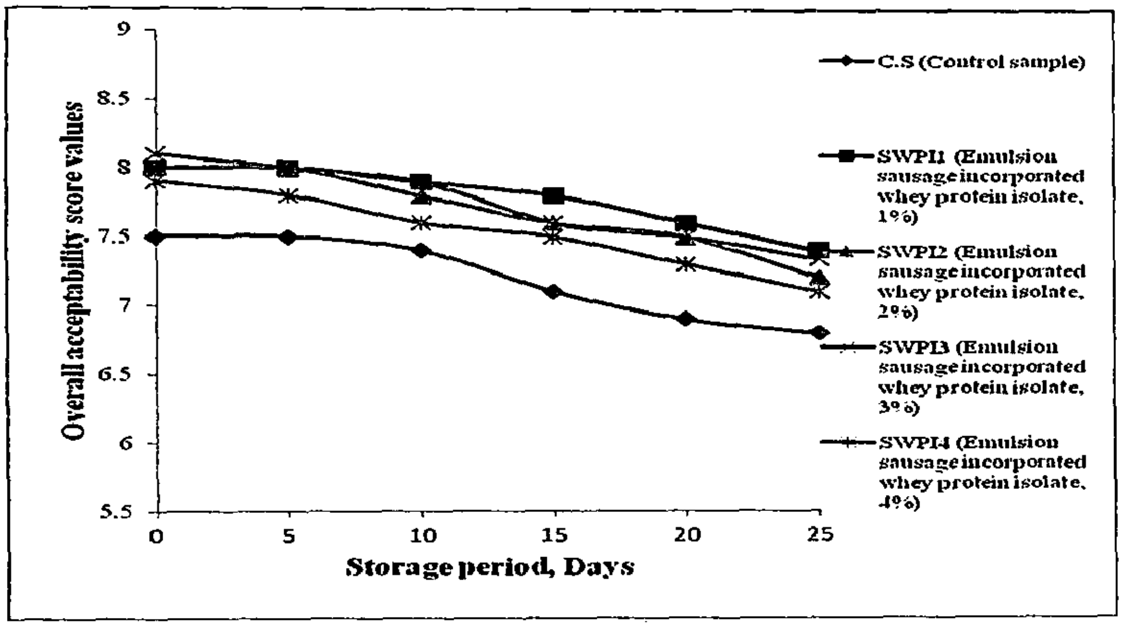



**Fig 4.109:** Regression analysis of overall acceptability of emulsion sausages incorporated with different levels of whey protein isolate during refrigerated storage (0°C)

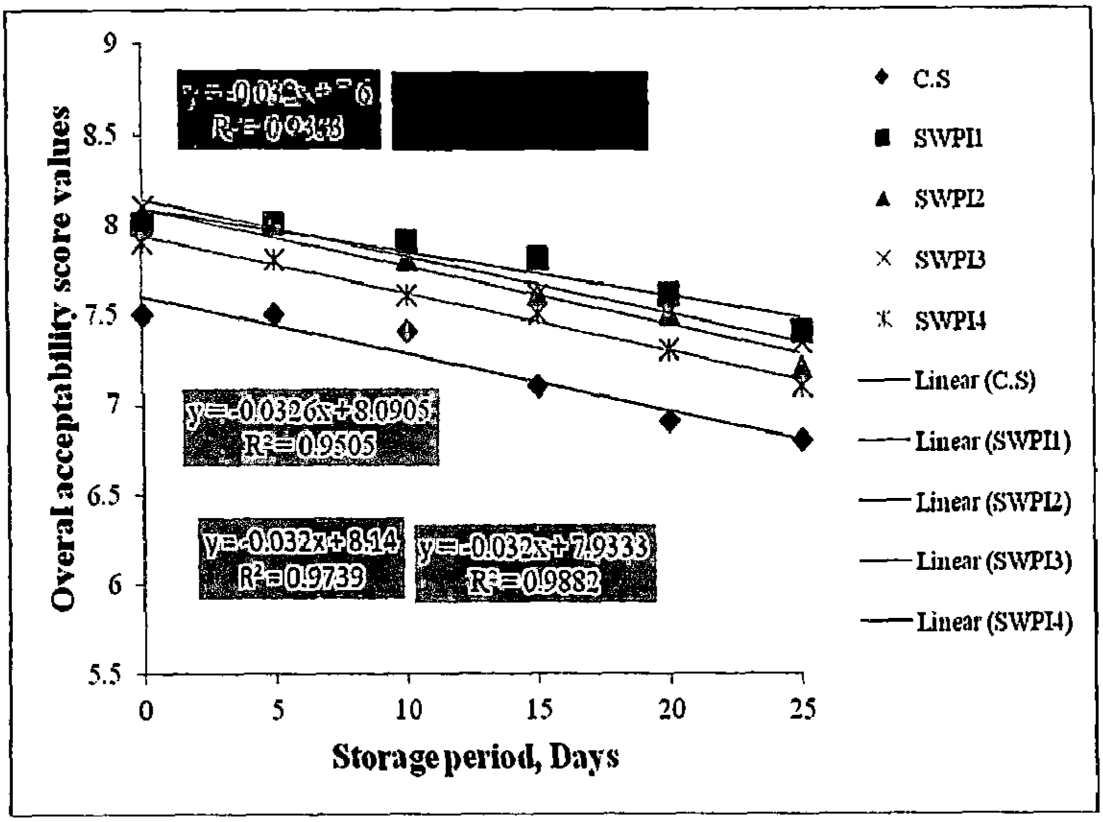


**Table 4.126:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein powder

| Sample Code | Overall acceptability score values | | | | | |
|---|---|---|---|---|---|---|
| | Storage Period (Days) | | | | | |
| | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | 7.5± 0.28 | 7.5± 0.21 | 7.4± 0.28 | 7.1± 0.22 | 6.9± 0.63 | 6.8± 0.72 |
| $Swpp_1$ | 8.2± 0.02 | 8.1± 0.25 | 7.8± 0.54 | 7.4± 0.28 | 7.0± 0.28 | 6.8± 0.53 |
| $Swpp_2$ | 8.1± 0.01 | 8.0± 0.71 | 7.7± 0.14 | 7.4± 0.49 | 6.9± 0.14 | 6.7± 0.43 |
| $Swpp_3$ | 8.2± 0.01 | 8.0± 0.70 | 7.9± 0.29 | 7.4± 0.37 | 7.3± 0.17 | 6.9± 0.08 |
| $Swpp_4$ | 8.0± 0.05 | 7.9± 0.63 | 7.8± 0.54 | 7.5± 0.35 | 7.2± 0.20 | 6.9± 0.10 |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

**Table 4.127:** ANOVA of overall acceptability of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage

| Source | DF | SS | MSS | F value (calculated ) | F value (tabulated 5%) |
|---|---|---|---|---|---|
| Total | 149 | 50.69302 | 0.340222 | | |
| Replicate | 4 | 1.905649 | 0.476412 | 2.742167 | 2.46 |
| FA | 4 | 3.452816 | 0.863204 | 4.968489 | 2.46 |
| FB | 5 | 27.10944 | 5.421889 | 31.20768 | 2.3 |
| Comb(A*B) | 29 | 1.54104 | 0.053139 | 0.305863 | 1.63 |
| Error/Res | 107 | 18.58972 | 0.173736 | | |
| LSD | 0.521963 | | | | |

DF = Degree of freedom, SS = Total sum of deviation square, MSS = Mean sum of deviation square, LSD = Least significance difference, FA = Different levels of whey protein incorporated, FB = Refrigerated storage, Com (A*B) = Combined treatment & storage

**Fig 4.110:** Effect of refrigerated storage (0°C) on overall acceptability of emulsion sausages incorporated with different levels of whey protein powder

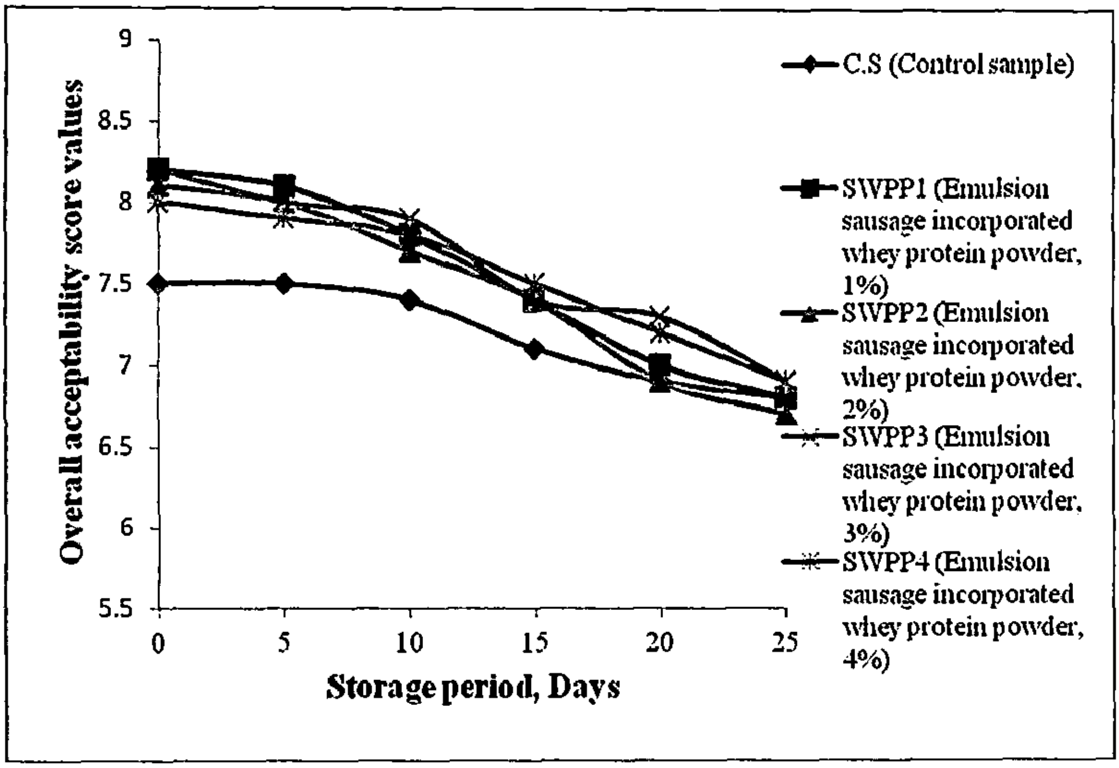
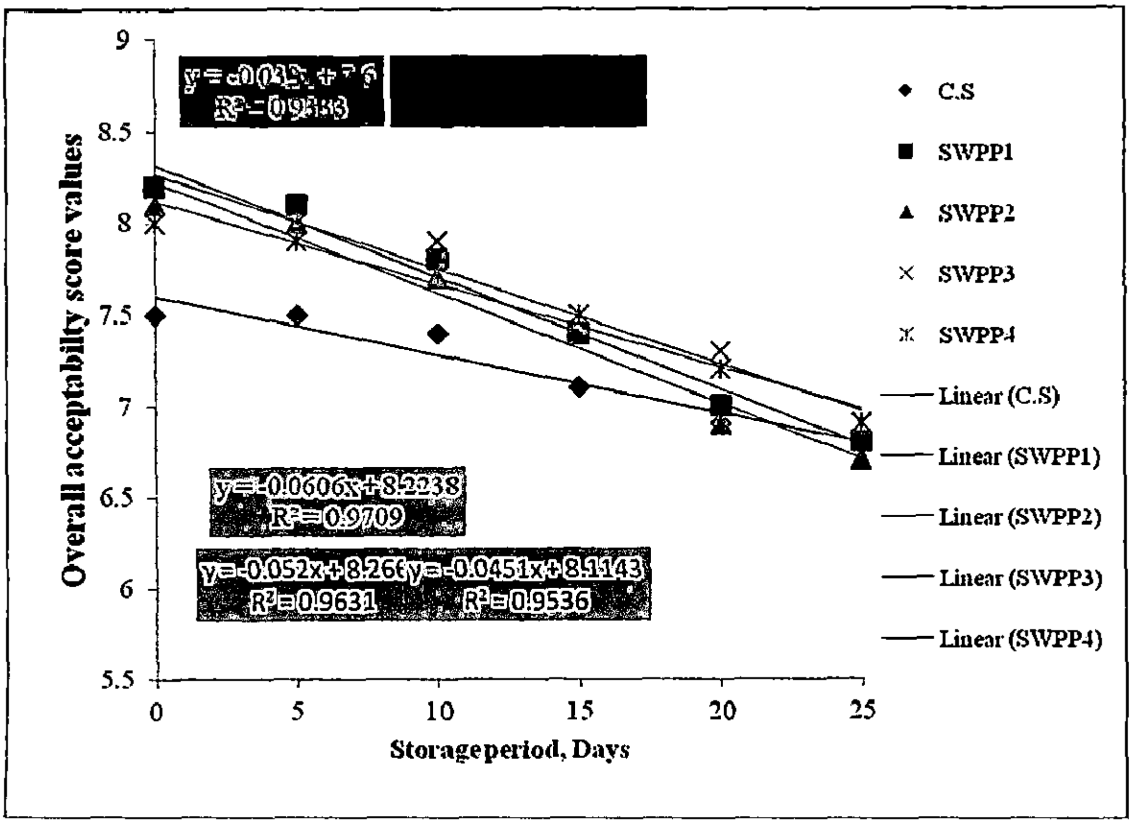



**Fig 4.111:** Regression analysis of overall acceptability of emulsion sausages incorporated with different levels of whey protein powder during refrigerated storage (0°C)



#### 4.2.9 Instrumental measurement of colour

Colour measurements of emulsion sausages incorporated with different levels of whey protein product (concentrate, isolate and powder) were also done by Hunter Lab. The instrument describes the colour in three dimensional system indicating value, hue and chroma (L, a and b). Value refers to lightness/darkness and it distinguishes light colour from dark or white from black. Maximum brightness is reported as 100. Hue is the colour attribute by which an object/food is judged to red, yellow, green, blue and so forth. It actually describes the shade of red/yellow/green/blue. The four basic colours are given maximum numerical value as 60. Chroma refers to vividness, depth, purity and saturation of a particular colour. It describes how much a particular object/foundation is yellow/ red/green/blue.

Table (4.38, 4.39 and 4.40) presents the results of colour evaluation of emulsion sausage incorporated with whey protein concentrate, isolate and whey protein powder respectively. The measurements of colour of samples were done in fresh condition and during refrigerated storage while sample was packed in combination film. The numerical value of 'L' in 25 days of storage samples condition found in the range of 18.86-27.77, 16.11-23.39 and 21.35–24.62 for emulsion sausage incorporated with whey protein concentrate, isolate and powder respectively.

The sample emulsion sausage incorporated with whey protein concentrate at the end of 25$^{th}$ day storage had 27.77% of maximum lightness as compared to 72.23% darkness. It was brown red colour in appearance. Hue values were found in the range 4.78–6.74. This indicated that maximum red colour was 6.74 as compared to yellow colour 'Chroma' value were found in the range of 6.43–9.24. This value describes the saturation of red colour, assuming maximum saturation at 60. L and b values were found to decrease dramatically during refrigerated storage (0° C) while 'a' values of first decreased and then increased on storage (0°C). The emulsion sausage lost its colour during refrigerated storage at end of 25 day. It was indicated by Hue values of the samples.

**Table 4.128:** Effect of refrigerated storage (0°C) on color of emulsion sausages incorporated with different levels of whey protein concentrate measured by Hunter lab

| Sample Code | | Instrumental measurement of colour | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage Period (Days) | | | | | |
| | | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | L | 33.22 | 32.43 | 23.75 | 18.27 | 27.83 | 24.89 |
| | a | 3.9 | 4.15 | 3.4 | 3.16 | 4.55 | 5.49 |
| | b | 9.22 | 9.27 | 7.73 | 5.26 | 8.06 | 6.28 |
| $Swpc_1$ | L | 31.23 | 22.98 | 24.07 | 23.88 | 28.23 | 27.77 |
| | a | 4.92 | 3.56 | 5.8 | 6.12 | 7.67 | 6.74 |
| | b | 8.78 | 6.27 | 7.03 | 7.56 | 7.79 | 9.24 |
| $Swpc_2$ | L | 30.28 | 20.97 | 19.92 | 22.99 | 24.67 | 25.06 |
| | a | 4.91 | 3.55 | 5.25 | 5.76 | 6.47 | 5.80 |
| | b | 8.85 | 6.36 | 7.91 | 7.11 | 7.77 | 6.70 |
| $Swpc_3$ | L | 29.50 | 26.69 | 28.32 | 20.75 | 27.42 | 18.86 |
| | a | 4.36 | 3.92 | 7.22 | 5.54 | 7.24 | 4.78 |
| | b | 8.4 | 7.86 | 9.57 | 6.78 | 8.14 | 6.43 |
| $Swpc_4$ | L | 30.37 | 32.55 | 25.27 | 25.25 | 26.92 | 25.02 |
| | a | 4.53 | 5.09 | 6.06 | 6.51 | 6.6 | 4.15 |
| | b | 8.80 | 9.60 | 8.57 | 8.13 | 7.98 | 7.70 |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpc_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein concentrate (1, 2, 3 and 4%)

**Table 4.129:** Effect of refrigerated storage (0°C) on color of emulsion sausages incorporated with different levels of whey protein isolate measured by Hunter lab

| Sample Code | | Instrumental measurement of colour | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage Period (Days) | | | | | |
| | | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | L | 33.22 | 32.43 | 23.75 | 18.27 | 27.83 | 24.89 |
| | a | 3.9 | 4.15 | 3.4 | 3.16 | 4.55 | 5.49 |
| | b | 9.22 | 9.27 | 7.73 | 5.26 | 8.06 | 6.28 |
| $Swpi_1$ | L | 23.17 | 23.19 | 18.48 | 24.74 | 23.46 | 16.11 |
| | a | 4.60 | 3.58 | 3.09 | 4.31 | 3.81 | 3.11 |
| | b | 4.72 | 6.84 | 5.29 | 5.47 | 7.00 | 5.42 |
| $Swpi_2$ | L | 22.31 | 18.98 | 24.67 | 20.21 | 22.78 | 22.12 |
| | a | 6.08 | 6.22 | 5.36 | 5.89 | 3.94 | 3.85 |
| | b | 6.17 | 7.30 | 8.26 | 5.95 | 7.66 | 7.46 |
| $Swpi_3$ | L | 22.72 | 22.56 | 19.12 | 24.94 | 22.54 | 23.39 |
| | a | 4.26 | 5.05 | 3.71 | 4.30 | 3.72 | 4.24 |
| | b | 6.57 | 7.46 | 6.25 | 5.98 | 7.44 | 7.20 |
| $Swpi_4$ | L | 25.07 | 25.91 | 19.03 | 26.13 | 25.61 | 22.61 |
| | a | 5.24 | 4.43 | 3.03 | 4.00 | 4.37 | 3.26 |
| | b | 7.45 | 8.45 | 6.11 | 6.67 | 8.22 | 7.02 |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs = Control sample, $Swpi_{1,2,3,4}$=Emulsion sausage incorporated with different levels of whey protein isolate (1, 2, 3 and 4%)

The samples of emulsion sausage incorporated with whey protein isolate at the end of 25$^{th}$ day of refrigerate storage had 23.39% of maximum lightness as compared to 76.61% darkness. The sample of sausage was brown red colour in appearance. Hue values were found in the range 3.81-5.96. This showed that maximum red colour was 6.08 as compared to yellow colour 'Chroma' value were found in the range of 6.34-7.79. This value describes the saturation of red colour, assuming maximum saturation at 60. L, a and b values were found to decrease during refrigerated Storage (0°C). The emulsion sausage incorporated with whey protein isolate lost its colour at the end of 25$^{th}$ day of refrigerated storage.

The sample of emulsion sausage incorporated with whey protein powder at the end of 25$^{th}$ day of storage had 24.62% of maximum lightness as compared to 75.38% darkness. It was brown red colour in appearance and Hue values were found in the range 3.81-5.96. This showed that maximum red colour was 5.96 as compared to yellow colour 'Chroma' value were found in the range of 6.34–7.79. This value described the saturation of red colour, assuming maximum saturation at 60. L, a and b values were found to increased during refrigerated Storage (0°C). Hughes *et al.* (1998) reported the addition of whey protein increased the lightness and decreased both the redness and yellowness values. Similarly Sammel and Claus (2003) advocated that lightness of ground turkey increased with the addition of whey protein powder. Moreover Youssef and Barbut (2011) stated there was no significant different in lightness and redness values on emulsified meat batters prepared at different protein levels. Researchers like Meltem and Eylem (2004) stated that sample with addition of dairy ingredients had no effect on $a^*$ values but into lower $b^*$ values.

**Table 4.130:** Effect of refrigerated storage (0°C) on color of emulsion sausages incorporated with different levels of whey protein powder measured by Hunter lab

| Sample Code | | Instrumental measurement of colour | | | | | |
|---|---|---|---|---|---|---|---|
| | | Storage Period (Days) | | | | | |
| | | 0 | 5 | 10 | 15 | 20 | 25 |
| Cs | L | 33.22 | 32.43 | 23.75 | 18.27 | 27.83 | 24.89 |
| | a | 3.9 | 4.15 | 3.4 | 3.16 | 4.55 | 5.49 |
| | b | 9.22 | 9.27 | 7.73 | 5.26 | 8.06 | 6.28 |
| $Swpp_1$ | L | 20.49 | 20.14 | 24.34 | 23.46 | 30.37 | 23.94 |
| | a | 4.61 | 3.98 | 5.07 | 5.50 | 6.55 | 5.96 |
| | b | 6.09 | 6.48 | 7.17 | 5.64 | 9.44 | 7.79 |
| $Swpp_2$ | L | 20.57 | 19.19 | 18.50 | 20.08 | 27.79 | 24.62 |
| | a | 3.35 | 3.65 | 4.02 | 4.47 | 5.73 | 5.48 |
| | b | 4.96 | 6.37 | 5.74 | 4.52 | 8.09 | 7.87 |
| $Swpp_3$ | L | 19.33 | 13.60 | 21.33 | 21.49 | 26.95 | 21.35 |
| | a | 3.99 | 2.12 | 3.46 | 4.67 | 4.98 | 3.95 |
| | b | 5.2 | 5.21 | 6.11 | 5.05 | 7.94 | 6.34 |
| $Swpp_4$ | L | 20.76 | 23.39 | 20.21 | 19.79 | 23.94 | 21.81 |
| | a | 3.85 | 4.26 | 3.90 | 4.19 | 4.32 | 3.81 |
| | b | 5.41 | 7.18 | 6.28 | 4.49 | 7.07 | 6.66 |

Values are mean of five replicates ±SD; Means with different letters in a column differ significantly ($p<0.05$), Cs= Control sample, $Swpp_{1,2,3,4}$= Emulsion sausage incorporated with different levels of whey protein powder (1, 2, 3 and 4%)

# CHAPTER-5

# *Conclusion*

# CHAPTER-5

# CONCLUSION

The studies were conducted to determine the effect of incorporation of whey protein products namely whey protein concentrate, isolate and whey protein powder on quality and shelf life of buffalo meat emulsion sausage. Incorporation of whey protein product makes important nutritional and quality improving contribution in the product of emulsion sausage.

Whey protein products have important quality improving effects on the meat products. It improves texture by modifying the binding capacity and helping in proper emulsification as the whey protein have a little quality of phospholipids in their composition. Whey proteins are globular molecules with a substantial content of a-helix motifs, in which the acidic and hydrophilic amino acids are distributed in a fairly balanced way along their polypeptide chains. Taking these functional and nutritional properties of whey protein products viz. whey protein concentrate, whey protein isolate and whey protein powder, it was incorporated in emulsion sausage.

The quality of sausages samples were evaluated on the basis of physico-chemical, microbiological, Sensory characteristics and colour measurement by Hunter Lab. Data obtained from experimental observation (n=5), were subjected to analysis of variance. The following conclusions were drawn for the study:

Whey protein is a complete protein and contains all essential amino acids and low in lactose content. Whey protein concentrate contain low levels of fat and carbohydrates. Whey protein isolate are processed to remove fat and lactose and contain at least 90% protein. Whey protein powder is containing highest quantity of fat and lactose among the whey protein concentrate and whey protein isolate. Whey proteins helped to improve muscle protein synthesis and growth of lean tissue of mass.

Whey protein products namely whey protein concentrate, isolate and whey protein powder incorporated with buffalo meat brought considerable improvement in quality characteristics of emulsion sausages samples.

Incorporation of whey protein products on buffalo meat emulsion sausage have a pleasant taste, excellent flavor and increased juiciness. Also product contains bioactive ingredients like immuglobulins and lactoferrin, which help to support the immune system. Emulsion sausages incorporated with whey protein products have rich abundance of branched chain amino acids and its quick absorption rate. Such products are important to help repair and rebuild muscles. Emulsion sausage treated with whey protein products have fresh, natural taste and do not contain isoflavones and any other component with potential hormonal effects.

Incorporation of whey protein products on buffalo meat emulsion sausage increased the protein content, moisture content, ash content, water holding capacity and extract release volume of emulsion sausage samples. pH values and fat content emulsion sausages decreased with treatment of whey protein products. The incorporation of whey protein products caused a little change in TBA number and total plat count of emulsion sausage.

Quality of emulsion sausages samples was evaluated during refrigerated storage (0°C). Refrigerated storage decreased moisture content, pH, water holding capacity and extract release volume of emulsion sausages samples, while the remaining properties (protein content, fat content, ash content and TBA number) significantly ($p<0.05$) increased in duration of storage.

Refrigerated storage significantly ($p<0.05$) increased total plate count, yeast and mold count and coliform count of emulsion sausages treated with whey protein products at 0°C. On 25$^{th}$ day of refrigerated storage (0°C) total plate count of all samples were found to be in the safe limit (3.32-3.93 log cfu/g), yeast and mold count was found to be less than 4 log cfu/g and , coliform count was found to be in the range of 2.16-2.54 log cfu/g. *Salmonella shigella* was not detected in all samples of emulsion sausages at all during refrigerated storage at 0°C till 25 days.

Emulsion sausages treated with whey protein products were acceptable to the panelist. All the fresh emulsion sausages incorporated with whey protein concentrate, isolate and powder with different levels (1, 2, 3 and 4%) had score values of sensory in rage of 8–9. Refrigerated storage decreased the sensory score values of emulsion sausage samples.

From the above results it was concluded that the emulsion sausage remained stable for 25 days at 0°C. It was reflected from microbial count and TBA number of the products. The product was liked by the panelist; it had enough nutritional value and was found to be safe for human consumption.

Colour measurements of emulsion sausages incorporated with different levels of whey protein products (concentrate, isolate and powder) were also done by Hunter Lab. The measurements of colour of samples were done in fresh condition and during refrigerated storage while sample was packed in combination film. The numerical value of 'L' in 25 days of storage samples condition found in the range of 18.86-27.77, 16.11-23.39 and 21.35–24.62 for emulsion sausage incorporated with whey protein concentrate, isolate and powder respectively. The sample emulsion sausage incorporated with whey protein concentrate at the end of 25$^{th}$ day storage had 27.77% of maximum lightness as compared to 72.23% darkness. It was brown red colour in appearance. Hue values were found in the range 4.78–6.74. The samples of emulsion sausage incorporated with whey protein isolate at the end of 25$^{th}$ day of refrigerate storage had 23.39% of maximum lightness as compared to 76.61% darkness. The sample of sausage was brown red colour in appearance. Hue values were found in the range 3.81-5.96. The sample of emulsion sausage incorporated with whey protein powder at the end of 25$^{th}$ day of storage had 24.62% of maximum lightness as compared to 75.38% darkness. It was brown red colour in appearance and Hue values were found in the range 3.81- 5.96.

The whey protein products are lighter in colour therefore they do not import any undesirable colour in meat product like emulsion sausage. As the presence of meat bring a brown or grey colour after cooking. The incorporation of whey protein products diluted this colour and brought to a very acceptable form.

Hence it is suggested from the study that incorporation of whey protein products makes important nutritional contribution in the meat products which would be liked and accepted by consumer. Such products can be commercialized and even they may target a particular group of the society as sportsman, wrestler, etc.

# RECOMMENDATIONS

- Buffalo meat can be replaced by poultry which is cheaper in those countries where buffalo meat is costly like gulf countries.
- The work on reduced fat can be under taken and whey protein products may be incorporated as water binding agent.
- Diabetic patient may be suggested to consume buffalo meat emulsion sausage incorporated whey protein isolate.
- Some natural antioxidant like rosemary, cinnamon and pomegranate (peel and seed extracts), etc, may be gives treatment in the products to increase the shelf life.

# CHAPTER-6

# References

# CHAPTER - 6

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# Publications

# PUBLICATIONS

(A) Research and Review Papers in International Journals.

(B) Chapters Published in the Springer's Book (New York).

  (i) Name of the Chapter: Meat Products and Byproducts for Value Addition

  (ii) Title of the Book: Food Processing: Strategies for Quality Assessment

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(C) Papers Presented International Conference.

# Effect of Incorporation of whey Protein Concentrate on Quality Characteristic of Buffalo Meat Emulsion Sausage

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**Abstract:** Incorporation of whey protein concentrate (WPC) at a level of 1, 2, 3 and 4% in buffalo meat was investigated for production, quality characteristic of emulsion sausage (ES). Quality of emulsion sausage was evaluated in terms physicochemical characteristics like moisture content, pH, protein content, water holding capacity (WHC), Extract release volume (ERV), microbiological characteristics like total plat count (TPC) and Yeast and mold count, and sensory characteristic including instrumental colour measurement. It was found that moisture content, protein content, ERV and WHC were significantly ($P<0.05$) increased in emulsion sausage as result of incorporation of 1-4% of WPC. Addition of 1-2% WPC decreased the pH. However 3-4% addition of WPC caused no change in pH and it remained comparable the pH of the control sample. TPC of ES was found in range of 6.23-6.37 log cfu/g. Emulsion sausage incorporated with whey protein concentrate was acceptable to the panelist. The numerical value of 'L' for the samples in fresh condition found in the range of 29.50%- 33.25%. Thus sample had 33.25 % of maximum lightness as compared to 66.75% darkness. The sample of fresh emulsion sausages was greyish brown colour in appearance.

**Keyword:** Sausage, Whey, Buffalo, Meat, Quality characteristic.

## INTRODUCTION

Consumers have demand a meat product which is safe, nutritious, convenient and attractive. By this approach meat industry in recent year is aimed to develop healthier meat products. Buffalo meats are cheaper compared to other type of meat. It is better to convert buffalo meat into value added products such as sausage. Sausage is a popular and highly relished meat product recognized world over [1]. The increase in meat production gave rise to various problems in handling, processing, preservation and storage, marketing and distribution till it reaches to the consumer table. These problems can be solved through research and development and the person involved in the meat industry need significant training to improve their traditional skill. Sausage formulations were designed by experts who, based on their experience were able to obtain the desired properties for sausage [2].

Buffalo meat can be very well used for production of sausage, a ready to eat and serve product. Sausage is a food that is prepared from comminuted and seasoned meat and is usually formed into a symmetrical shape. Whey proteins are by-products of the cheeses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Nowadays, great efforts are being made to find new uses for whey proteins, e.g. production of edible film [3]. Whey protein improves emulsion stability, provide better colour properties and result in lower chewiness and elasticity [4]. Whey and whey products are used in sausage to improve the flavour, taste, texture and increase the protein of finished products. Sweet whey, whey protein concentrate has 34-80% protein and whey protein isolate has more than 90% protein. These products are among the most common whey products used in processed meats [5].

Quality improvement, nutritional optimization and cost effectiveness are key drives in using whey protein ingredients in processed meats. The addition of WPC to meat products an increase of WHC, with the result of reduces of the cooking weight loss. This addition also improves sensory quality and enhances nutritional values of meat products [6]. The addition of WPC had a lesser influence in the total yield when the NaCl concentration was higher than 1.6% (w/w) when The sous vide cooking pasteurization protocol was 70 °C–2 min at the slowest heating point of muscles [7].

Andres *et al.* [8] reported chicken sausage with a total lipid content in the range of (0.22%, to 6.09%), with increasing WPC decrease the sausage hardness and cohesive was obtained. Low-fat chicken sausages incorporated with xanthan and guargums and WPC had good acceptable sensory scores and functional properties [8]. Exhaustively washed chicken breast muscle improved by the addition of WPC in the emulsion stability heated cream layers [9]. The addition of 2% of WPC in Bockwurst sausage, the sausage had higher stability than with 0.00, 1.75, or 3.75% addition

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# Effect of whey protein concentrate on quality and shelf life of buffalo meat emulsion sausage

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**Abstract:** Incorporation of whey protein concentrate (WPC) at a level of 1, 2, 3 and 4% in buffalo meat was investigated for production, quality characteristic and shelf life of buffalo meat emulsion sausage (ES). Quality of emulsion sausage was evaluated in terms physicochemical characteristics like moisture content, pH, protein content, water holding capacity (WHC), Extract release volume (ERV), microbiological characteristics like total plat count (TPC) and Yeast and mold count (Y&M), Coliform count, and sensory characteristic including instrumental colour measurement. It was found that moisture content, ERV, pH and WHC were significantly ($P<0.05$) decreased of emulsion sausage in period of storage at 0° C as result of incorporation of 1-4% of WPC. But protein content significantly ($P<0.05$) increased in period of refrigerate storage. TPC, Y&M and Coliform count of emulsion sausage were found in range of 7.11-7.39 log cfu/g, 3.34-3.86 log cfu/g and 2.25-2.54 log cfu/g respectively. Emulsion sausage incorporated with whey protein concentrate was acceptable to the panelist. The numerical value of 'L' for the samples in 25$^{th}$ days of storage condition found in the range of 25.02%-32.55% Thus sample had 32.55% of maximum lightness as compared to 66.45% darkness.
**Keywords:** Whey protein concentrate, Emulsion sausage, Incorporation, Sensory characteristic, Shelf life

## INTRODUCTION

Meat and meat product are nutritionally rich, providing a wide range of nutrients, such as protein, fat, minerals and vitamins and constitute an important part of the European diet [1]. Meat has long been considered a highly desirable and nutritious food, and has become a mass consumer product throughout the world with the highest consumption rates being recorded in industrialized Western countries. Meat and meat products make an important nutritional contribution to the diet of the people. A significant percentage of the recommended dietary allowances for proteins, vitamins-B, magnesium, iron and zinc are contributed by red meat and poultry [2]. Indian buffalo meat production is growing significantly. Although no official production statistics are available, industry sources and export data indicate that continued strong export demand is triggering an expansion in buffalo meat supplies in India. As a result, new slaughterhouses are emerging, providing farmers with a new market for non-productive buffalo heifers, bulls and bull calves. Calendar year (CY) 2013 Indian buffalo meat production is thus forecast to rise to a record 4.16 million tons (on a carcass weight equivalent basis), up 14 percent from CY 2012. CY 2012 buffalo meat production is estimated at 3.64 million tons (up 12% from CY 2011), and CY 2011 production has been slightly revised up to 3.24 million tons [3]. Buffalo meat can be very well used for production of sausage, a ready to eat and serve product. Sausage is a food that is prepared from comminuted and seasoned meat and is usually formed into a symmetrical shape. Sausage making had developed over a number of countries, beginning simple process of salting and drying meat. This was done to preserve fresh meat that could not be consumed immediately. The typical flavors, texture, and shape of the many sausages known today, such a frankfurter, braunschweiger, and salami were named due to the geographical location of their origin. The emulsion sausages cook sausages that have been finely comminuted to the consistency of a fine paste. Hot dog, Frankfurter, Mortadella, Bologna, liver sausage are typical examples. Emulsion sausage will be successful if the enough lean meat has been selected and enough myosin has been extracted. The lean meat is the main source of myosin. The more myosin extracted, the thicker and stronger protein coat develops around particles of fat. In cause of myosin depends on how vigorous the cutting process was and how much salt (and phosphates) was added. Whey proteins are by-products of the chesses making industry and have generally been disposed of as animal feed or used in infant formulas and sports food. Now a day, great efforts are being made to find new uses for whey proteins, e.g. production of edible film [4]. Whey protein improves emulsion stability, provide better

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*Research article*

# EFFECT OF INCORPORATION OF WHEY PROTEIN POWDER ON QUALITY CHARACTERISTIC OF BUFFALO MEAT EMULSION SAUSAGE

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**BSTRACT:** Investigations were carried out to study the effect of incorporation of different levels of whey protein owder (1, 2, 3 and 4%) on quality characteristics of buffalo meat emulsion sausage (ES) in fresh condition. The quality f ES was based on physicochemical characteristics namely moisture content, ash content, fat content, protein content, BA number (Thiobarbituric acid), extract release volume (ERV), water holding capacity (WHC) and microbial haracteristic viz total plat count (TPC), yeast and mold (Y&M), coliform count, salmonellashingella and sensory haracteristic including instrumental colour measurement. The moisture content, protein content, ash content, TBA umber, ERV and WHC were significantly ($P<0.05$) increased in emulsion sausage samples as result of incorporation ith 1-4% of whey protein powder (WPP) as compared to control sample. However fat content of emulsion sausage ignificantly ($p<0.05$) decreased. TPC of ES was found in range of 3.70-3.93 log cfu/g. The numerical value of 'L' for ie samples in fresh condition was found in the range of 19.33%- 20.57%.

**(ey words:** Emulsion sausage, Whey protein powder, Extract release volume, Water holding capacity, Physicochemical characteristic

## NTRODUCTION

Vhey proteins are by-products of the chesses making industry and have generally been disposed of as animal feed or sed in infant formulas and sports food. Now a day, great efforts are being made to find new uses for whey proteins, e.g. roduction of edible film [1]. Whey protein improves emulsion stability, provide better color properties and result in ›wer chewiness and elasticity [2]. Whey protein consists of a number of individual protein components. The two most bundant proteins are β-Lac (50-55%) and α-Lac (20-25%). β-Lac has a molar mass of 18.3 kDa and diameter of about mm. The isoelectric point is 5.1 and the denaturation temperature is 78°C. β-Lac is largely responsible for solubility, elation, foaming, emulsification, and flavour binding of whey protein. Because β-Laos is the most abundant protein in 'hey, it has been suggested to be one of the main determinants of the properties of whey protein gels [3]. Consumer emand healthier meat products that are low in fat, salt, cholesterol, nitrates and calories in general and contain in ddition health-promoting bio active components such as carotenoids, unsaturated fatty acids, sterols and fibers on the ther hand, furthermore, consumer accept these level meat products with altered formulations to taste, look and smell ie same way as they're traditionally formulated and processed counterparts. Emulsion sausage incorporated with whey rotein powder is one product, which can be prepared from buffalo meat using other ingredient salt, spices, condiment, 'hey protein (isolate, concentrate, whey powder), fat and animal fat. The product will have a pleasant taste, excellent avor and increased juiciness. This study was designed to determine whether whey powder at the levels (1, 2, 3 and %) added in the formulation of emulsion sausage prepared with buffalo meat would help to improve the quality haracteristic of sausage.

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REVIEW ARTICLE

# Development in sausage production and practices-A review

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## Abstract

In this review development of sausage production practices and effects of incorporation of the different non-meat ingredient on meat products specially meat sausages were discussed. The beneficial effects of added ingredients *viz.* vegetable protein, whey protein, herbs, fibers and spices in meat products especially meat sausages were discused along with quality of meat products. Further, the addition of non-meat ingredients not only improve the quality of the meat products, but also reduce the cost and have beneficial health effects on consumers.

**Keyword:** Sausage, meat products, processing, non-meat ingredient, whey protein, vegetable protein.

## Introduction

Increasing consumer demand for quality meat products results in the development of meat products by incorporating health enhancing ingredients. The long standing positive consumer perception that meat and meat products are the best sources of minerals, vitamins and complete proteins (complete proteins are those proteins contains all of the amino acids that our body needs to function properly) (Verbeke *et al.*, 2010). Selection of meat for sausage production is important in achieving good quality products. All the formulas of sausage production are based on meat and also all additives used in sausage production must be based on weight rather than percentage.

Consumer demand healthier meat products that are low in fat, salt, cholesterol, nitrates and calories in general and contain in addition health-promoting bioactive components such as carotenoids, unsaturated fatty acids, sterols and fibres on the other hand; furthermore, consumer accept these level meat products with altered formulations to taste, look and smell the same way as they are traditionally formulated and processed counterparts. At the same time competition is forcing the meat processing industry to use the increasingly expensive raw material i.e. meat more efficiently and produce products at lower costs (Jochen *et al.*, 2010).

The non-meat ingredients are used in meat products to improve the quality and reduce the cost of the products. These ingredients of very wide sources such as dairy, eggs, plants and microbial including probiotics are incorporated in these meat products (Xiong, 2012; Yadav *et al.*, 2013). These additives able to increase nutritional value, consumer acceptability and benefits to human healths. Table 1 showed the some sausage incorporated with different non-meat ingredient.

## Sausage Production: Ingredients and Raw Materials

For thousands of years people have prepared meat products similar to today's sausage. Whoever spoke glowingly of sausage in the Odyssey, saying it was a favorite food of the Greeks. Roman festive occasions were considered incomplete without it. Sausage is a food product resulting from the assemblage of proper ingredient in the right proportion coupled with a structured design and controlled process. The quality of the product is always a reflection of the status of the raw materials and the process. If the process has been carefully designed and developed then the raw materials have to standard to achieve the expected level of quality. Also every raw material for sausage should making measure to the requirement of the quality of the sausage. Every raw material should given specification to details of all important criteria and service that affect the quality of the product. It should define all physical, chemical and microbiological requirements.

Fresh and high quality meat such as lamb, beef, pork, mutton and poultry also cuts from head and leftover cuts may be used. Other ingredients such as salt (addition to taste, extract some proteins from meat, enhances flavor, reduce microbiological spoilage and

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REVIEW ARTICLE

# Buffalo: a potential animal for quality meat production-a review

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## Abstract

India has a major show in world buffalo population; Pakistan and China are second and third in the world of the buffalo population. Indian buffalo meat productions are growing significantly. Buffalo meat is one of the healthiest meats among red meat for human consumption; it is low in calories and cholesterol. Buffalo meat is dark red in colour; it is because of less intramuscular fat or more pigmentation. The dark meat possesses good binding properties and is preferred in product manufacture. Buffalo meat can be very well used for production of sausage, a ready to eat and ready serve product. The quality and quantity of buffalo meat depend on many factors, the most important of which are the water buffalo type and breed, age, feeding intensity, management system and environmental conditions. This article presents a review on the buffalo meat production and the physicochemical properties of buffalo meat.

**Keyword**: Buffalo, meat, production, nutritional value, physicochemical properties

## Introduction

Buffalo is one species being seen today as a saviour animal to meet man's increased requirements of food in the coming times. India and Pakistan are home to the best buffalo breeds in the world. India has a major show in world buffalo population; Pakistan and China are second and third in the world of the buffalo population. India has 105.1 million and they comprise approximately 59 percent of the total world buffalo population, which Pakistan and China have respectively 29.0 million and 23.27 million. They can find buffalo in more than 50 countries of the world, varying in ecology, climate and topography. Buffalo has been the main of the rural economy of farmers in many developing countries (Ramesh, 2013). Buffaloes are of two types, riverine buffalo and swamp buffalo. Riverine buffaloes are distributed in India, Pakistan, Sri Lanka and some European countries such as Italy, Bulgaria, Greece and Yugoslavia. Swamp buffaloes are spread in Far East Asia, including China, Indo- China, Indonesia, Philippines and Thailand (Ross, 1975).

The world buffalo population is estimated at 185.29 million head, spread in some 50 countries, of which 179.75 million (97%) are in Asia. During the last 10 years, the world buffalo population increased by approximately 1.49% annually, by 1.53% in India, 1.45% in Asia and 2.67% in the rest of the world (Antonio and Marco, 2005). India is the first country in Asia for scientific and technological development in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement. The production of buffalo meat has high growth compared to beef production. The quality and quantity of buffalo meat depend on many factors, the most important of which are the water buffalo type and breed, age, feeding intensity, management system and environmental conditions. Buffalo meat is the healthiest meat among red meats known for human consumption because it is low in calories and cholesterol. It has almost 2-3 folds cost advantage over mutton and goat meat. Several studies have demonstrated that buffalo meat has higher iron proportions than other species, protein content and low fat values becoming a raw material of high potential for industry (Cedres, 2002). Buffalo product presents healthy physicochemical characteristic respect to beef product, especially in terms of protein, fat and iron contributions against securities (Eyas *et al.*, 2007).

Indian buffalo meat production is growing significantly. Although no official production statistics are available, industry sources and export data indicate that continued strong export demand is triggering an expansion in buffalo meat supplies in India. As a result, new slaughterhouses are emerging, providing farmers with a new market for non-productive buffalo heifers, bulls and bull calves. Calendar year (CY) 2013 Indian

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Review Article

# SAFETY AND RISK MANAGEMENT IN MEAT AND MEAT PRODUCT MANUFACTURE BASE FOR APPLYING HACCP

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## ABSTRACT

Providing of food hygiene for all food processing as a part of approach of food hygiene management, to reduce the incidence of food borne disease is the need of an hour. Training is important in raising industry awareness about hazard Analysis Critical Control Point (HACCP) which ensures the potential benefits of HACCP to the food industry, regulatory authorities and ultimately to the consumer. HACCP provides a means to identify and assess potential hazard in food production and establish preventive control procedures for those hazards. The application of HACCP is actively encouraged and usually required to all of the food supply chain. In meat processing we must create a high level of qualified potency in the safety of the product produced by a processing system both by individual processors as well as processors operating under the system of control. Meat processing is generally a process step to prevent and reduce hazards to safe levels by applying the HACCP.

**Keyword:** HACCP, Food safety, Hazard, Food hygiene management, Meat processing

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# PHYSICOCHEMICAL STUDY OF PET FOOD DEVELOPED FROM BUFFALO MEAT BY- PRODUCTS

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## ABSTRACT

The meat processing industries contain a lot of buffalo meat by-products as waste materials which create several health hazards and environmental pollution. India rank first in buffalo meat in the word. The by products of buffalo meat contain vitamins and minerals, which makes it functional and nutritional. Investigations were carried out to study the effect of incorporation of tripe meal and rice flour on development, quality evaluation and storage stability of pet food under ambient condition. The quality of pet food was based on physicochemical characteristics namely moisture, ash, fat, protein content, pH, The Analysis using paired sample t-test for optimization. There was moisture content (8.211%), Ash content (3.571%), protein (17.85%), pH (6.688) and fat in the range (14.323%). The analysis model was found significant for protein, pH, ash and were not significant for moisture and fat.

**Keyword:** Pet Food, Tripe meal, Rice flour, Bone meal, Paired sample t-test.

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# Quality evaluation and shelf life studies of pet food developed from broken rice, buffalo tripe and bone meal

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## Abstract

Investigations were carried out to study the effect of incorporation of tripe meal and rice flour on development, quality evaluation and storage stability of pet food under ambient condition. Different levels of tripe meal (50-55%) and rice meal (40-45%) were incorporated while other ingredients like bone meal were added in the same ratio in all twenty one samples. The quality of pet food was based on physicochemical characteristics namely moisture, ash, fat, protein content, pH, TBA number (Thiobarbituric acid) and microbial characteristic viz TPC (total plat count). The RSM (Response surface methodology) was applied to study the effect of tripe meal, rice flour and other ingredients and for optimization. During ambient storage moisture content of pet food samples was found to increase slightly. There was apparent decrease in fat and protein content of pet food samples during ambient storage mainly due to the increase in moisture content. There was slight decrease in pH of pet food sample during ambient storage. They were stored for 180 days.

*Keywords:* Pet Food, Tripe meal, Rice flour, Response surface methodology.

## Introduction

In recent years, the pet food industry has become a multimillion dollar business all around the world. In developing countries such as Turkey, this industry is located at the top point, with the large amounts of both local and imported products available for the consumer. There are several types of pet food available for cats and dogs that include dry foods, wet foods, canned foods, moist foods, semi-moist foods, frozen-chilled pet foods, and treats. Pet food generally consists of meat, meat byproducts, cereals, grain, vitamins, and minerals. Dogs are carnivores and their digestive system is by 2/3 shorter than that of pigs. Therefore, the nutritive components have to be of high quality, rich in energy, and easy to digest, in particular the vegetable components. The present contribution discussed results of studies investigating the effects of extrusion processing on the physicochemical and microbiological quality of dry pet foods and provides

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# Meat Products and Byproducts for Value Addition

**Saghir Ahmad and Abdol Ghafour Badpa**

## 1 Introduction

Meat refers to skeletal muscle and associated fat and other tissues, but it may also describe other edible tissues such as offal. Conversely, meat is sometimes also used in a more restrictive sense—the flesh of mammalian species (pigs, cattle, lambs, buffalo, etc.) raised and prepared for human consumption, to the exclusion of fish and other seafood, poultry, and other animals.

The word meat comes from the Old English word mete, which referred to food in general. The term is related to mad in Danish, mat in Swedish and Norwegian, and mature in Icelandic and Faroese, which also mean food. The word "mete" also exists in Old Frisian (and to a lesser extent, modern West Frisian) to denote important food.

Meat is defined as the edible portion of the muscles of terrestrial animals, particularly cattle, sheep, pigs, etc. In practice this definition is restricted to a few dozen of the 3,000 mammal species, but it is often widened to include edible organs like kidney, liver, brain, and heart as well.

Adult mammalian muscle flesh consists of roughly 75 % water, 19 % protein, 2.5 % intramuscular fat, 1.2 % carbohydrates, and 2.3 % other soluble non-protein substances. These include nitrogenous compounds such as amino acids and inorganic substances such as minerals (Lawrie and Ledward 2006; Hodgson et al. 2006). Muscle proteins are either soluble in water (sarcoplasmic proteins, about 11.5 % of total muscle mass) or in concentrated salt solutions (myofibrillar proteins, about 5.5 % of the mass) (Lawrie and Ledward 2006).



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# Management of Food Safety Based on Applying HACCP

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In recent years consumers have importance emphasis on food safety and food free from diseases such as heart disease or cancer. In food safety should prevent of hazards in foods such as physical, biological and chemical hazard. A HACCP program can be implemented for control of physical, biological, and chemical risks throughout operations. The plan helps assure regulating authorities and customers that you are taking every reasonable precaution to assure food safety. It also helps you reduce contamination-related food losses and improve the design of new food products. HACCP has the most important to bring the safety plan successful. Hazard is bringing risk if it crossing the limit of critical controlled point. Hazard has been different tieted in relation to free safety and food quality. It is associated with its producing and its intended use. HACCP is a system which identified, evaluates and control hazard which are significant for food safety. HACCP examine every step in food operation, identifying specific hazard as well as implementing effective control measures and verification procedures. Hazard Analysis Critical Control Point is not a zero risk system, it's designed to minimize the risk and as such risk management tool. Hazard Analysis and Critical Control Point, It is a preventive approach implemented by industry to control food safety hazards. Using HACCP principles during inspections will help to assist you in evaluating the effectiveness of food safety management systems implemented by industry.

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# NUTRITIONAL VALUE AND SHELF LIFE OF PROCESSED FOOD

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Food processing is the conversion of agricultural produce to some suitable form. Food processing involves to several step namely primary, secondary and tertiary processing of agricultural crops. When the crop is harvested from field, basic unit operation like dehusking, winnowing and separating are, perform to get refined raw material. Secondary processing is further performing for the conversion of shape of natural produce. Tertiary processing organised in food industry. The industries like bakery industry, snake foods industry, baby food industry and many other industries. In which the food products of high nutritional value and sufficient shelf life are processed. Food safety rules are applied in during processing to prevent the food from hazard (Physical, Chemical, microbiological). The HACCP applied in food industry to safe from farm to fork. During processing some nutrient lost. For example thermal processing leads to 70% loss vitamin C as the result of heat processing. They processed food is fortified after processing in other to maintain in natural food values. Consumers are interested to purchase processed food with high nutrition value, good taste, zero health risk and good shelf life.